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Is the long recession at an end?

After nearly a decade of deepening recession, it is natural to hope that rising stock markets mean the end is near. But it is too soon to cheer. Too many structural weaknesses persist.

Hurrah, the long recession is coming to an end. That is the simplest interpretation of what has happened in the past few weeks on the international stock markets, Wall Street in particular. For if people are willing once again enthusiastically to invest in the shares of industrial corporations instead of lending to their governments, does that not imply that confidence in the future is flowing back? That the corporations whose shares are being snapped up will now adjust their research and development plans to encompass projects not directed at short-term goals? That the same corporations will return to the labour market for scientists and engineers? And that the rising tide of prosperity that the ending of the recession could bring may permit and even encourage governments to relax the parsimonious strings by which basic research and university research in particular have for too long been constrained? Hopeful speculation along these lines is inevitable but, unfortunately, also premature.

Prudent scepticism springs from several sources. What has happened in the past few weeks on Wall Street has happened before. The flurry of excitement about the stocks of industrial corporations in August this year, in retrospect the first step in the upward movement of stock prices that has now carried the Dow Jones index of industrial stocks to more than 1000, was quickly followed by a trend in the opposite direction. The same could happen now, but probably not precipitously; eagerness to buy shares in industrial corporations has spread beyond the financial institutions to ordinary people wondering what to do with their savings, thus generating momentum that will not quickly be dissipated. But last week's peak on Wall Street is not in any case the Everest that it seems. The index had not then managed to exceed the value of 1051 that it reached in 1973, on the eve of the first dramatic increase of the international price of oil. That is a measure of how far the value of industrial enterprises has been depressed by the long recession. Yet now, it seems, potential investors are declaring with their pocket-books that they believe that prosperity is round the corner. Will they be proved right?

The signals are contradictory. The biggest plus is the decline of the inflation rate in the United States, from close on 15 per cent three years ago to an estimated 4-5 per cent by the end of this year. This is striking proof of the flexibility of the US economy, and that the monetarist monetary policies followed by the Federal Reserve Board in the past two years have been effective. Elsewhere, similar policies have worked less quickly. In Britain, three years of the Thatcher government's version of monetarism preceded by at least a year of Labour government's restraint have produced a less dramatic reduction of inflation (which may nevertheless turn out to be only 6 per cent by the end of this year). No wonder, in the circumstances, that the dollar remains for the time being strong. The minuses, unfortunately, are also memorable. The chief of these is that in the United States the federal government is still with its eyes open running a budget deficit of more than \$100,000 million, the consequences of which are more than ever likely to be inflationary now that the Federal Reserve Board has decided (last week) to encourage a downward trend of interest rates. For to the extent that people with money to invest (savings, in other words) are now less likely to be attracted by lending to the government (which may partially account for the high jinks on Wall Street), the Department of the Treasury may have to resort to the printing press to keep the government in

business. That could quickly crimp Wall Street's enthusiasm.

Three more deeply seated worries cry out. First, throughout the industrial world except (for special reasons) Japan, unemployment has risen to heights unprecedented for half a century. In the United States, unemployment amounts to more than 10 per cent of the potential workforce. In Britain, the magic figure of 3 million unemployed has long since been passed, but the dole queues are still lengthening. Even once ebullient countries such as West Germany are learning to live with unemployment (while France is following the 1960s practice of trying to make the problem go away by public spending sustained by external devaluation and borrowing from the international banks). The trouble is that much present unemployment will persist (and be a public charge) unless governments are much cleverer than in the past at fitting badly trained or untrained people for the world that lies ahead. Second, and closely linked, is the glaring truth that even the hardships of the past few years have not made industrial and service enterprises in the West into the super-productive outfits they could be. British motor car manufacturers, for example, still compete poorly with those elsewhere except in their wage rates (which is hardly a cause for boasting). This points to one respect in which the recession as so far run is incomplete, and the third shadow over the next few months: many of the corporations whose shares have been bought in the past few days are so dependent on bank loans that they may never be able to repay, and in the circumstances (such as low wage rates) peculiar to the middle of a recession, they may not be able to survive the glare of whatever light there may be at the end of the tunnel. The international banks, with their mountain of doubtful debts still counted as assets in their balance sheets, are very vulnerable.

For the time being, none of this seems to weigh with those with money to invest. The Federal Reserve Board may have made lending to the government unfashionable, but there is no reason to suppose that the huge flow of cash down Wall Street will immediately be converted into radical innovations that will sustain some future era of prosperity. Although investors seem marvellously to have been persuaded that manufacturing industry has a future, manufacturers themselves are noticeably more cautious. The explanation is not hard to find. In the usual economic cycle of boom, bust, recession and recovery, fortunes are made in the first two cycles and stand to be lost (as stands to reason) in the second two. The trouble, this time round, is that the structural problems of industry (public as well as private) persist. There has been an ample harvest of bankruptcies (which will continue) but nothing yet to suggest that some brave new world is about to be inhabited. Governments for the past few years preoccupied with the passive enforcement of monetary restraint must now take a more emphatic view. Briefly, in the interests of their own survival (and of the well-being of those who vote them into office) they must put their money where their mouths are, and must invest in the only window on the future they are allowed by their constitutions to occupy — the exploration of what the future is like, and how to live with it. But that means research and education, surely? Surely. So governments in Washington and elsewhere should give up trying to second-guess Wall Street, which is itself a symptom and not a cause, and themselves intelligently begin investing in the intangible commodities that may help to ensure that there is a future for all of us.



Paris asks more of academics

Protests over productivity demands

The French university system is about to be taken by the scruff of the neck and given a good shake, just as it was in the student revolution of 1968. The difference is that this time the government will be doing the shaking, and the objective is not so much student democracy as economic relevance.

To some left-wing university lecturers, the plans of education minister Alain Savary, outlined last week to the council of ministers and to university presidents, have been something of a rude awakening. Savary wants more students, more work from the university staff, and courses more closely linked to the jobs students might take in the outside world; and all this by October 1984 and with no real increase in university budgets. It is being said that only a socialist government could dare to be so firm. Giscard d'Estaing's minister Alice Saunier-Seïté was hounded for little more.

Savary's weapon is to be a new law for higher education, which will be put before the national assembly in the spring of 1983. This will be the first reorganization of the university system since Edgar Faure's law of 1968, which regrouped the previously autonomous "faculties" into multi-disciplinary universities, gave students some constitutional power, and paved the way for the foundation of many new — and often very small — universities throughout France. Faure's law was said to be little more than a pacification of the rebels: it was thought out too hastily to last.

Savary's law, then, will tidy up the Faure reform, unlike Saunier-Seïté's plans, which were more to reject the reform altogether. Savary wishes to retain open entry to the first year, and indeed to increase the number of students by attracting more from the working class. He wants the first two years to be broadly orientated towards a profession, and the last year clearly so. He wants university staff to teach for longer hours, pay greater attention to their students and play a greater part in community life; and he wants better links between the universities, the independent university institutes of technology (which give a strictly professional training) and the prestigious *grandes écoles* (which produce top engineers, managers and the higher echelons of the civil service). He will also make it easier for universities to make profitable links with industry, in an attempt to encourage innovation, as the minister of research and industry tried to do for the research councils. Thus the whole tendency is to

open the universities to the economy.

So far, university staff have responded most strongly to the proposals for more students and more work. There are already so many students in the first year that most have to be turned away at the first selection — which comes for entry to the second year. The students who come and go in the first year are called the "ghost students". Does Savary want to create more ghosts, they ask? As for more work, the staff claim they are already overworked.

At the ministry, however, the universities are seen somewhat differently — as wastrels and spendthrifts. Already the director for higher education and research at the ministry, M. Jean-Jacques Payan, recently arrived from the director-generalship of the Centre National de la Recherche Scientifique, has addressed a letter to the universities ordering an extension of the university year from 25 weeks to 32, and proposing a reduction (by 20–25 per cent) in the number of hours of part-time teaching to be allowed next year. This will increase the teaching load for full-time staff without increasing pay. For some lecturers who are required to teach only

three hours a week this may hardly be burdensome; but for others who already teach far more than their statutory requirement it will lead to resentment. Savary's problem may be to distinguish between those parts of the universities that really require reform (often non-scientific) and those that are already doing a good job (often scientific).

However, Savary is concerned with the broad mass of higher education in his law, not science itself. So there has been no mention in his statements of plans to alter the French higher degree system of a *troisième cycle* (2 years postgraduate work and a thesis) and the *thèse d'état* (somewhat beyond a PhD and presented at the age of 30 or so). The omission does not indicate that the plans have been dropped, however. Chevènement introduced reform of this system — essentially to an America-style PhD — in his law for research, and Savary supported the innovation in the National Assembly. Jean-Jacques Payan also supports it, and so it is still expected to find its way into the Savary law next spring.

Robert Walgate

Swedish crisis: keeping tryst

Sweden's Social Democratic Party was returned to power last month with an election platform that included a major commitment to science, higher education and technical research. The first actions of the new government, however, were to devalue the krona and to announce the guidelines of a new "crisis programme" to combat inflation and boost public spending on social welfare (particularly housing and full employment). The programme, embodied in a bill to go before Parliament at the beginning of November, envisages a substantial drop in the standard of living. It should not, however, necessarily curtail the election platform on science and technology because many of the targets then urged by the Social Democrats have been reiterated by the crisis programme.

Both promise and programme urge the need for better use of the country's mineral and timber resources especially on energy. Indeed, according to the new Finance Minister, Mr Kjell-Olof Feldt, the crisis programme was largely necessary because Swedish industry is too energy-intensive and has been badly hit since oil prices rocketed in the 1970s. Thermal pumps, solar heating and hydroelectric power all featured prominently in the Social Democratic platform. The restructuring of the country's energy resources is particularly urgent, because, as a result of a national referendum, Sweden is committed to phasing out nuclear power within twenty-five years.

Similarly, although the tax concessions granted by the previous government to stimulate private investment in industry are to go, the crisis programme, according to Mr Feldt, would permit continued expansion in fields of investment likely to promote the future competitiveness of industry. This accords with the election platform which urged the establishment of a state structural development fund to help investment projects that would otherwise be difficult to finance. Election proposals for greater state participation in industrial research, the introduction of new scholarships for inventors and increased research grants to colleges of technology all fit in with the "crisis" investment strategy.

The reduction of unemployment is a major aim of the crisis programme. To some extent, young Swedes have tried to escape the dole queue by enrolling in higher education. University officials report a record number of applications this year, in many cases clearly motivated by the fact that one prefers to say one is a student rather than that one is unemployed. Moreover, the Swedish educational system is particularly convenient for such enrolments. Tuition is free in all Swedish universities and (in spite of cuts by the previous administration), there is a fairly extensive programme of financial aid in the form of a small non-returnable grant plus an interest-free loan (but with the principal pegged to inflation) repayable from one's future earnings, over up to 20 years. Moreover, the "democratization" of Swedish univer-

sities embodied in the Higher Education Act of 1977 encourages the recruitment of adult students, while the syllabus structure facilitates the staggering of courses over a longer time-scale to accommodate, for example, family commitments. It is not

only school-leavers, therefore, who are resorting to the universities, according to the Stockholm daily *Svenska Dagbladet*, more than half the students in Stockholm are now over thirty years of age, and nearly a quarter of them over forty. **Vera Rich**

Social science research

Debates go on

The British Social Science Research Council (SSRC) will not, after all, be disbanded but instead will have its budget cut. Sir Keith Joseph, the Secretary of State for Education and Science, announced earlier this week that he had decided to reduce by £6 million the recommendation of the Advisory Board for the Research Councils (ABRC) that SSRC should have £73 million to spend in the three years from next April. By doing so, he reminded everybody who is the boss, but has also ensured that arguments will continue about the functions of advisory committees whose advice is rejected before it is made public.

The SSRC budget has become a contentious issue in the desultory argument about the support of British academic research. Just over a year ago, Sir Keith triggered off an argument about academic autonomy by announcing that he would cut £500,000 from the present annual budget of SSRC and that he had asked Lord Rothschild to inquire into its functions and to report. The subsequent discovery (by means of private letters between Sir Keith and the Chancellor of the Exchequer published by *New Society*) that Sir Keith was unsympathetic to social science research seems not to have cast him down.

Sir Keith was at pains this week to emphasize that his decree is less generous than the Rothschild recommendation (in May this year) of a frozen budget (in real terms). He has also asked the council to consider changing its name and to shift more of its support for graduate students (now running at 45 per cent or £8.8 million per year) from quotas awarded to university departments to awards to students who would then choose where to study.

Earlier this week, Sir Keith insisted that social problems were important, that SSRC spent only one-eighth of the government's total expenditure in this field but that the social sciences differ from the natural sciences in that the results of social science research are not "falsifiable". "You cannot prove that a scientific theory is correct, but you can carry out experiments to prove it wrong."

On balance, SSRC is likely to be relieved by the way things have turned out. By some accounts, even after the publication of Lord Rothschild's recommendation that the council should be kept going, SSRC's chairman Mr Michael Posner has spent a summer of disquiet, said on Monday that he did not question Sir Keith Joseph's right to countermand ABRC's advice, but that he thought Sir Keith had been influenced by people "who try to have it both ways".

The next instalment in this saga will be on 27 October, when ABRC is to be allowed to publish its latest advice to the British government. ●

Nuclear safety

Action soon on thermal shock

Washington

The US Nuclear Regulatory Commission (NRC) may soon act to require eventual modifications in older pressurized water reactors to reduce the risk of potentially disastrous ruptures in the reactor vessel. A recently completed study by the staff of NRC's Advisory Committee on Reactor Safeguards (ACRS) showed ten plants that may need such modifications between now and the year 2000. At least some of these plants may be forced to make a choice between very expensive modifications or closing before their originally anticipated useful life is up.

A rupture can occur when the steel reactor vessel, which in older plants tends to become brittle as a result of exposure to radiation, is quickly cooled while kept under pressure. This would happen if a small break in the primary cooling system (which runs through the vessel and then a heat exchanger to produce steam in a secondary loop) occurred and cold emergency core cooling water were pumped in, or similarly if emergency feedwater were pumped into the secondary system because of a leak there. In either case, the water in the primary loop would cool off quickly while the pressure remained close to its normal high level. As the inside of the 8-inch thick walls of the vessel cooled, the resulting stresses could aggravate an existing flaw, resulting in a surface crack; the high internal pressure could then drive the crack completely through the vessel wall. In the worst case, the core cooling water would be lost and the core would melt.

Although this problem of "pressurized thermal shock" has been recognized for some time, two recent discoveries have fuelled concern. Test results from samples placed in reactors have shown over the past few years that the embrittlement from radiation is proceeding faster than previously thought. The second discovery came in an analysis of the 1978 Rancho Seco accident (in which a partial power failure in the instrumentation caused a valve in the steam generator to be opened, leading to overcooling of the core) which concluded that if the plant had been older, a rupture could have occurred.

How much cooling it takes to make a crack grow in the reactor vessel depends on a property of the metal referred to as its reference temperature. This is the temperature at which the vessel, when cooling, changes rapidly from ductile to brittle behaviour. As the reactor vessel gets older

— that is, as it is exposed longer to radiation — the reference temperature climbs.

The ACRS staff has recommended no absolute cut-off on acceptable reference temperatures; rather, they have proposed a "screening criterion". When the welds (the critical spots, because they contain a small amount of copper) that run along the length of the vessel reach a reference temperature of 130°C, or the welds that run around the circumference reach a reference temperature of 150°C, a more thorough analysis of the plant would be made to determine what should be done. This criterion was established because of the ultimate probability of a core melt. Operating experience suggests that an overcooling accident in which the temperature in the primary loop falls below 150°C can be expected once every 100 reactor-years. (The normal operating temperature of the fluid is 290°C.)

No reactors exceed the screening criterion yet, but five are expected to by 1988–1990.

The possible solutions proposed are reducing the radioactive flux reaching the reactor vessel by placing the older fuel elements on the outer ring of the core, modifying the control system automatically to depressurize the primary system as it cools, increasing the temperature of the emergency core cooling water and emergency feedwater, and even annealing the vessel to restore its ductility (which would require removing the core, drying out the vessel and inserting an electric heating grid to raise the vessel's temperature to 450°C for a week).

The ACRS staff recommendation does not propose any across-the-board solution, though. According to staff member Roy Woods, "it's a plant-specific problem". Three years before a plant would reach the screening level, it would have to submit a plan for approval by NRC.

Critics have been quick to point out the large uncertainties in the screening criterion, something that the ACRS staff concedes while maintaining that they have been conservative in their calculations. Robert Pollard of the anti-nuclear Union of Concerned Scientists said also that NRC has never systematically looked for conditions that could lead to pressurized overcooling. "The only part of the thing that is certain," he said, "is that if the vessel ruptures the emergency core cooling system can't prevent melting of the core. The only protection is preventing it from occurring". **Stephen Budiansky**

French research directorate

Twelve-year rule relaxed?

The "12-year-rule" by which French research directors would have to resign after 12 years in the job (see *Nature* 14 October, p.569) was defended strongly last week both by M. Jean-Pierre Chevènement, minister for research and industry, and by M. Pierre Lazar, the director-general of the medical research council INSERM where reaction has been strongest. But the rule has been relaxed with the result that some are left wondering exactly what it will achieve.

For example, Chevènement made it clear at a press conference last week that a director could take a sabbatical year at the end of his 12-year-term — and then be reappointed. Also, said Lazar, a research unit could be split into different parts, with the old director appointed director of one of the new parts, or others federated under new titles. Further, the rule refers to 12 successive years, and so may perhaps be construed to be restarted after a period abroad during the term. The rule is therefore much more elastic than had been made out.

But according to Professor Pierre Chambon, a rebel member of the INSERM directorate, "this achieves nothing". The whole exercise, he says "has been a waste of time and money; it wasn't any better under the previous government".

According to Chambon — and to many others — the real problem is one of evaluation: how to judge effectively the value of scientific research groups. In France, this is done by largely elected committees which have a large trade union representation. Although the unions have no direct control over the membership of committees, their influence is too strong, Chambon believes. "I know of no other example in the world where scientific judges are effectively chosen on the basis of union membership", he says.

Moreover, the committees are too open: they should be chosen on the basis of expertise, and should involve foreign experts, Chambon argues. In the present reform of the research councils, the government "had a unique chance to change the way the committees were appointed but they have made the situation even worse". The result was the invention of the arbitrary 12-year-rule — a period too long for the worst directors and too short for the best.

Lazar, in fact, agrees with Chambon in many respects — but he has had the responsibility of constructing a politically acceptable system. "The goal [of the 12-year-rule] is to have true scientific judgements" he said last week. "But we have such heavy structures in France, the idea of stability of employment is so deeply engrained, that we have to struggle to find ways to reach the goal."

Last year, said Lazar, INSERM received a proposal for only one scientific director

(out of 250) to be changed. "Something had to be done to make the system move." There was no major reason, said Lazar, for thinking that the new assessment committees would have changed their method of judgement. The 12-year-rule "is a very soft system". By contrast, making negative judgements on a scientific director of 10, 12 or 15 years standing was "very hard".

Moreover, "there will be no destruction of good research units". Lazar insisted. "We do not want to smash research!"

Tropical medicine

WHO programme blessed

The World Health Organization (WHO) Special Programme on Research and Training in Tropical Diseases has done well during its first five years of operation, according to the report of an external review committee set up by Dr D.E. Bell of the Harvard School of Public Health at the instance of the WHO Council. But the special programme could face increasing difficulties if donors do not increase their financial contributions as field trials for drugs and vaccines make new demands.

The special programme was set up in 1976 to develop ways of controlling six major tropical diseases — malaria, schistosomiasis, filariasis, trypanosomiasis, leishmaniasis and leprosy — by supporting research in established institutions and by strengthening new institutions in developing countries.

Five years later, the six diseases are still prevalent, and some even appear more common. The external review committee says, however, that the explanation may be more efficient identification of the diseases, and thus indirectly a mark of the success of the special programme. The committee's report says that although no single disease has yet been conquered, significant progress has been made, for example, towards a leprosy vaccine and the use of *Bacillus thuringiensis* as a biological agent for the control of vectors. It is estimated that the programme now accounts for about 25–30 per cent of all research on the six diseases.

The system of awarding research grants, along the lines adopted by national research councils, has its strengths and weaknesses, partly recognized in the review committee's report. It is cheaper than building new institutions and allows new people, perhaps working in disciplines not traditionally thought relevant to tropical diseases, to be brought in when appropriate. But the report does not mention the dangers of relying heavily on the infrastructures of established research institutions which may then come under threat from national research budget cuts. The

There has been a 6 per cent increase in jobs at INSERM this year, and a 30 per cent increase in budget.

There is also conflict between the medical profession and research interests on this issue, said Lazar. This is why there had been an "explosion" over the matter at INSERM but hardly a whimper from the non-medical Centre National de la Recherche Scientifique.

Too many INSERM research directors also hold hospital jobs and teaching jobs at universities, Lazar thinks. When a person becomes a research director, his other duties should be "lightened". Becoming a research director is not a matter of gathering titles.

Robert Walgate

London School of Hygiene and Tropical Medicine is a prime example (see p.671).

The report does, however, complain that the research grant system, which is run internationally, is too dependent for its success on excellent central coordination and management. The committee recommends that the operation of the academic steering committees which decide on the allocation of grants should be streamlined, and that greater care should be taken to avoid conflicts of interest when members of the steering committees apply for grants.

The committee also says that the special programme should be reviewed again in five years, when a judgement can be based on its record of research. Meanwhile, the committee points out that after a rapid initial increase the programme budget has declined in real terms since 1979 from \$21.7 million to an estimated \$17.3 million (1979 prices) in 1982.

Contributions have come from the United Nations Development Programme, WHO, the World Bank (since last year), charities, trusts and about 25 national governments of which the largest contributors have been Denmark, Sweden and the United States. Britain, which has received nearly 11 per cent of the money spent on research grants since the start of the special programme, has not paid up in the past two years. The British Overseas Department Administration, doubtless embarrassed, has, nevertheless said that it will resume contributions from 1983–84, but at a lower rate than the previous \$800,000 a year.

One solution canvassed by the committee is to ask the pharmaceutical industry as well as national governments for money. The programme has already established links with industry, which is expected to benefit by manufacturing and marketing the fruits of research, and this year signed one of the first agreements with a company, the Wellcome Foundation, to develop new drugs against filariasis. As products reach the stage of field trials, however, the strain on the budget will increase.

Judy Redfearn

UK universities

Ex-premier attacks Thatcher

Mr Edward Heath, the former Conservative Prime Minister, fresh from his denunciation earlier this month of government economic policy, last week broadened his attack on his party's government to the field of higher education. Speaking at the London School of Hygiene and Tropical Medicine, he condemned the present government's preoccupation with ridding the public purse of the cost of education from public funds. "That", he said, "is not what education is about."

Proudly declaring himself a heretic, Mr Heath said that it is a tragedy that the London School of Hygiene and Tropical Medicine, as a consequence of government policy, is being denied the funds to do its job properly. The school seems to have been especially hard hit this year. Overseas students at the school, 20 per cent up last year, have not this year materialized as hoped, leaving the school with a shortfall of £265,000. And the school is the only part of the University of London whose grant has been cut this year, by 3 per cent in cash terms or by more than 10 per cent allowing for inflation.

One of the school's immediate anxieties is the overhead cost of research projects, estimated as something like 30 per cent of the value of research. While the university's subvention as a percentage of

process of survival in the traditional community of an Indian village. Players participate as members of small family units who must manage their limited resources to produce and sell rice for their livelihood. International development institutions such as the World Bank and the Food and Agriculture Organization have apparently expressed interest in the game, which is marketed by Marginal Context Limited. Under the British government's small firms loan guarantee scheme, the company obtained a commercial loan from

the National Westminster Bank and has produced one thousand kits for sale at £195 a set.

As a former member of the Brandt Commission on North-South relations, Mr Heath spoke about the deteriorating situation of the developing countries. Referring to the Secretary of State for Employment, Mr Heath said: "Tebbit's doctrine of 'Get on your bike and get on with it' does not apply in Third World countries. It doesn't apply here either".

Jane Wynn

British telecommunications

Shares for all

The British telecommunications industry appears to have survived one of its most eventful weeks since the replacement of the cat's whisker by the triode in the 1920s. A committee under Lord Hunt, Secretary of the Cabinet in Mr Edward Heath's time, issued a liberal but tricky set of recommendations about the introduction of cable television to the United Kingdom. ITT agreed to dispose of a controlling interest in its British subsidiary, Standard Telephones and Cables Limited, whereupon the offer for sale of 40 million shares in the British company was promptly oversubscribed thirteen times. And the Department of Industry denied newspaper reports that the government intends to break its promise earlier this year not to sell off shares in the publicly owned telecommunications network British Telecom.

Politically, the Hunt report will preoccupy the British government in the next few weeks, at least until the new parliamentary session begins on 3 November. The issue to be decided is whether to set aside time for legislation, in the process giving the declared opponents of the Hunt report an opportunity to protest, or to embark on cabling Britain informally, with the consent only of British Telecom.

Whatever happens, the cabling of Britain cannot begin in earnest until next spring, after the report on 1 March from a committee set up by the Department of Industry to consider the standards that should be followed by the cable television system. The committee (under Dr E.N. Eden, previously in charge of British standards at the Department of Trade) will specify technical standards that must be met by interfaces between different cable systems, by interactive systems (in which users can answer back) and by direct broadcasting satellite systems (with which, in due course, cable television will have to compete).

The Hunt committee's report (Cmd 8679, HMSO), accepts without comment the British government's view that the rapid development of cable television would help to rescue the British economy from the doldrums. By recommending that holders of cable television franchises

Science books: Frankfurt gloom

Heidelberg

At the world's largest annual international book fair held in Frankfurt (FRG) (6-11 October) 5,688 publishers presented some 300,000 books and journals, including 86,000 new publications but there was a 22 per cent reduction in new scientific titles.

As 90 per cent of scientific literature is bought by libraries, this must reflect a worldwide downturn in library spending. In West Germany the outlook is grim. In June this year the West German UNESCO Commission called attention to the plight of the country's libraries. Throughout the 1960s and 1970s, West German universities were a fairly firm market for academic literature. But, in 1981 and 1982, cuts in library budgets (1982 average 19 per cent), together with steeply rising prices and the fall in the value of the deutschmark, have combined to reduce buying power of university libraries by 35-40 per cent.

In 1982, 80 per cent of all libraries had reduced budgets. In North Rhine Westphalia, for example, the budget cut was 62.5 per cent. Some libraries are buying no books this year and a national survey showed that, in 1981-82, 26,000 journal subscriptions were cancelled, at least 10,000 without substitution. Information gaps are arising that will be difficult and expensive to fill later.

Meanwhile the libraries are making strenuous coordinated efforts to economize. There is a trend to specialist collections, partly supported by grants from the Deutsche Forschungsgemeinschaft, to more interlibrary lending, and greater use of photocopying. The result for publishers is a downward spiral of increasing prices and decreasing sales. Expensive monographs and journals published on marginal budgets are particularly vulnerable. Specialist journals, on the other hand, with a clearly defined readership are safer.

Sarah Toozee



income has been falling, from 44 per cent three years ago to 30 per cent now, research income has been rising. Meanwhile, research grant income has increased from 40 per cent to 45 per cent of the total. The dean of the school, Dr C.E. Gordon Smith, said last week that he will now have to consider not taking on extra research grants. Because research income covers about 75 per cent of staff costs, this would have serious implications. A deficit for the year of around £300,000 is on the cards.

Mr Heath had turned up to launch a green revolution — the name of a game devised by academics at the school and the University of Cambridge as a teaching device for students and managers of agricultural development projects. The "Green Revolution Game" simulates the

should be virtually free from regulation, the report has excited the opposition of broadcasters and guardians of public morals. But franchises will last for only eight years, and may be withdrawn if operators persistently to renege on their promises or offend against public taste.

Conventional communications appear to offer more immediate returns. ITT's disposal of its controlling interest in Standard Telephones and Cables (STC), intended to rid the British company of the taint of being a subsidiary of an international conglomerate, appears to have been a chance for British investors to buy into success in high technology. The announcement early in February that STC is to leave the group of three manufacturers (including Plessey and GEC Limited) working with British Telecom on the development of the digital communications network known as System X may even have strengthened its hand in the stock markets.

The future of the company seems to lie in the development of terminal equipment for the national communications system made possible by the liberalization and intended sale of British Telecom. The point could be important because Standard Telephone Laboratories, one of the largest technical development laboratories in Britain, is a wholly owned subsidiary of STC. While some of the laboratory staff are still working on System X, the laboratory (where pulse-code modulation was invented) is at present recruiting people. ●

Teletext in Australia

Buying British

Canberra

British teletext has won an easy victory to be chosen as the standard system in Australia. On 26 September, the Minister for Communications, Mr Neil Brown, endorsed the UK teletext system for Australian television.

Teletext is a non-interactive (one-way) computer-based information retrieval system and is broadcast by being carried "piggy-back" on television signals. Teletext signals appear as a line of dots on the edge of the television tube not normally visible on the screen. With the aid of a decoder these signals are made intelligible as a page of text which flashes on the screen at the touch of a button. By contrast, a related system, videotext, which shares the same imaging system with teletext, is interactive (two-way) and is transmitted by means of a telephone line or by cable television. An existing telephone network is generally used to distribute or call up information.

The decision on a national teletext system has settled the question of standards, and local television stations can now go ahead and offer teletext services. These services will open up a new market for decoders and boost British industry —

French reactors

The French construction programme for nuclear reactors may be halved in 1984 and 1985, if budget-conscious elements in the government have their way.

The reason: a ballooning government deficit and borrowing requirement, immense debts of FF120,000 million (£10,000 million) at Electricité de France which must buy the reactors, and a substantial downturn in projections for electricity consumption in 1990 due to poor economic growth.

The previous government had proposed that a start be made on the construction nine reactors in 1982 and 1983; the present government eventually authorized six (much to the chagrin of the environmentalists). Now the target of six more for 1984 and 1985 would be reduced to three if the penny-pinchers won the day.

Almost half of French electricity production is nuclear. But the availability of the nuclear plants (pressurized water reactors built under licence from the US Westinghouse Corporation) is slipping below the world average. In June the working reactors were available only 49 per cent of the time, in July 50 per cent. The world average is about 60 per cent. On that basis, more reactors may be needed simply to keep up present production. **Robert Walgate**

Australian television sets have the same number of lines as British sets, so the same sort of decoder could be used with only minor modifications.

If and when a national videotext system is established, the system of choice is likely to be Prestel, because of the common graphics between British teletext and Prestel. At present the British systems are incompatible with the French Antiope or the Canadian Telidon systems, which gives Prestel an advantage.

In contrast with teletext services, a public videotext system seems some way off. Plans for Telecom to introduce videotext faded in October last year, when the then Minister for Communications, Mr Ian Sinclair handed the market over to the private sector. Telecom, in fact, had been ready to market Prestel. Telecom urged that while private videotext can service large organizations and businesses capable of supporting a network, it cannot provide a mass market with a service at reasonable cost. Since then there has been a suggestion that Telecom could provide gateways to various databanks but it may not be happy to provide that facility without a computer databank of its own. Meanwhile Prestel has secured an agreement with a small Melbourne-based company, Computerpower, to market the information held in Prestel's computers in Britain.

Vimala Sarma

Nobel prize

Structure of life

Aaron Klug of the Medical Research Council Laboratory of Molecular Biology in Cambridge has won this year's Nobel Prize for Chemistry. (For the Physics Prize see p.675.)

Klug first arrived in Cambridge as a graduate student from South Africa in 1949; 15 years later he went to Birkbeck College in London and he returned to Cambridge in 1962. At Birkbeck, Klug developed an interest in the structure of viruses and some of his first studies were on tobacco mosaic virus (TMV). At first he employed X-ray diffraction, collaborating with Rosalind Franklin — whose contribution to the solution of the structure of DNA he has always championed.

To supplement X-ray studies, Klug employed high resolution electron microscopy to produce models of the structure of helical and spherical viruses. A key concept to emerge from that work was the "principle of quasi-equivalence" of subunit packing of the protein particles of viruses. Don Caspar (now at Brandeis University in Massachusetts) and Klug put forward that principle in 1962.

After his return to Cambridge in 1962, Klug continued to devote much of his attention to TMV to understand further the interactions between protein and nucleic acids. Three classic papers on the assembly of the RNA and protein components of TMV into the functioning virus were published in *Nature New Biology* in 1971. And ever higher resolution structures of the TMV disc protein have continued to appear, the ultimate being the 2.8 Angstrom structure of 1978 (*Nature* 276, 362).

The Nobel prize citation for Klug's prize rightly is not confined to his contributions towards understanding the structure of complexes of proteins and nucleic acids, notably TMV but more recently also the nucleosome, the repeating structural unit of chromatin. Instead the citation is also for his development of crystallographic electron microscopy. That technique arose from Klug's realization that the diffraction methods of X-ray crystallography could be applied to electron microscopic images of regular biological structures. The technique emerged in the mid-1960s, at first using optical diffraction, but by 1968 Klug and David de Rosier were reconstructing three dimensional images by computer analysis of Fourier transforms of negatively stained electron microscopic images. Among structures to have yielded to Klug and these techniques have been tail fibre of bacteriophage T4, thin filaments of muscles and the nucleosome.

Others may have contributed as much as Klug to individual accomplishments but the Nobel committee has recognized that the whole is greater than the sum of the parts.

Peter Newmark

Environmental protection

US Congress finds fault

Washington

Congressional discontent with the Reagan Administration's continued failure to enforce environmental statutes brought an angry response last week from Environmental Protection Agency (EPA) officials, who argued that enforcement has actually increased in the most critical areas. EPA is responsible under several major environmental laws for monitoring industry compliance and referring violations to Justice Department lawyers for prosecution. The agency refers fewer cases for prosecution now than it did two years or more ago, according to an analysis prepared by the subcommittee on oversight and investigations of the House Energy and Commerce Committee, chaired by Representative John Dingell, (Democrat, Michigan). EPA referred 252 cases in 1980, but only 78 cases in 1981 (the first year of Reagan's term) and 91 in the first three-quarters of 1982.

The subcommittee also cited several other indicators of reduced enforcement.

- Federal inspections of hazardous waste facilities decreased from 1,825 in fiscal year 1981 to 1,006 for the first three-quarters of fiscal year 1982.
- EPA has not enforced a requirement that toxic spills be reported. It is receiving only 41 per cent of the anticipated number of such reports.
- EPA was budgeted in fiscal year 1982 for 318 inspections of chemical manufacturers to see that they were notifying EPA before beginning production of any new chemical. Only 30 inspections were performed, 29 in the last quarter.

Kirk Sniff, EPA's director of enforcement policy, said however, that these statistics do not tell the whole story. "We know the states have taken up a large part of the slack, particularly in pesticides and drinking water", he said. He conceded that EPA would not be sure of how effectively states were enforcing other environmental statutes until December, when an "enforcement indicator system" will be in operation.

Sniff also said there had been a change in the nature of the cases which in part explains their declining number. The major cases in 1980 and before, he said, were against plants that had not installed required pollution control equipment. That effort was largely successful, he said; now, the agency is faced with cases that are "worlds more difficult" against plants that fail to operate or maintain that equipment properly.

John Todhunter, EPA's assistant administrator for toxics and pesticides, accused the subcommittee of playing fast and loose with the agency's enforcement statistics. He said the "low" figure of 30 pre-manufacture notification inspections resulted from his decision to concentrate

on a more critical problem, that of polychlorinated biphenyls (PCBs). In fiscal year 1982, 1,715 PCB inspections were carried out, in comparison with the 850 budgeted for. Inspections in the toxics programme had doubled since the previous year, and even the 30 pre-manufacture notification inspections were a three-fold increase over 1981. "My enforcement people are madder than hornets" about the subcommittee document, he said.

Yet two of the subcommittee's accusations may be harder for EPA to refute. One is that EPA referred a curiously large percentage — 17 per cent — of its cases on the very last day of the 1982 fiscal year. (Of the hazardous waste cases, 45 per cent were referred on the last day.) This, the subcommittee said, may reflect an attempt to bolster enforcement statistics.

Sniff maintained that these cases "weren't things that were whipped up at the last minute" and denied that there was any quota for the staff to meet. He said the cases had been in the works for months; his

Soviet Agriculture

Poor harvests spawn new efforts

The Soviet Union faces major food shortages this winter. So much is clear from the recent admission, by agriculture minister Valentin Mesyats, that farm production this year has been disappointing. Although final figures have not yet been announced, the grain yield is expected to be in the region of 170 million tonnes, against a target of 240 million tonnes. Grain purchases are already being negotiated with the United States and a five-year contract with France is under discussion. Items in short supply include fruit, vegetables, dairy produce and meat, although meat supplies are liable to pick up later this autumn as peasants slaughter livestock because of the shortage of fodder.

The shortfall comes at the end of a summer which saw the launch of a new long-term food programme, hailed as one of the most important components of Soviet economic strategy for the next decade. Soviet agriculture, according to the plan, is to be reorganized on the basis of "agro-industrial complexes", a term nowhere defined but which seems to imply reorganization, planning and further mechanization.

The new food programme includes several major research projects in crop-breeding to produce disease-resistance, soil conservation and hydrology. Genetic engineering programmes which were previously orientated to pharmaceutical production have been switched to agriculture, and at least one new research institute, the Institute of Soil Science and

only explanation of the apparent rush to refer cases by the end of the fiscal year was that "our people all have internal deadlines and requirements" and that "everyone is assessed in terms of the fiscal year".

The other criticism that isn't likely to go away is that of EPA's assumption that increased enforcement by the states is compensating for reduced federal activity. The subcommittee memorandum notes, for example, that while state inspections did make up for the decline in EPA's inspections of hazardous waste facilities this year, they cannot be counted on to do the same in future, given the Administration's plan to reduce and eventually to eliminate federal hazardous waste grants to the states. According to the National Governors' Association, those funds now cover 69 per cent of the cost of the state hazardous waste programmes. The Administration's budget proposal for fiscal year 1983 (which has not yet been acted upon) calls for a 20 per cent cut in these grants and in grants for state air, water and drinking water programmes.

The Dingell subcommittee has scheduled hearings on the matter for early December.

Stephen Budiansky

Photosynthesis in Pushchino, has been established as part of the programme.

The Soviet public seems somewhat sceptical. In a broadcast last month dealing with questions from the public Polad Adzhievich Polad-Zade, a deputy minister of land reclamation and water resources, defended poor yields from the Soviet harvest in comparison with the United States and Canada, on the grounds of climate. Agricultural production is considered impossible without irrigation if the annual rainfall is less than 400 mm — yet 40 per cent of Soviet arable land falls into this category. With only 1 per cent of Soviet arable land suitable for farming without special attention, a guaranteed harvest, said Polad-Zade, is possible only with large capital investments in irrigation, land reclamation and the use of mineral fertilizers. Irrigation and land reclamation therefore play a major role in the Food Programme, which has taken over the plans to divert north-flowing rivers southwards into the Volga, Don and Kuban basins, first proposed during the 1920s and reactivated during the 1970s.

Even when these grandiose engineering works are accomplished, they will only exacerbate one of the main headaches of Soviet food planners — how to get the food to the centres of population. Waste and inefficiency in despatch of perishable foodstuffs is a constant target for criticism in the Soviet press. So the food programme includes many measures aimed simply at improving transport systems.

Vera Rich

CORRESPONDENCE

South African link

SIR — Unfortunately the Science and Engineering Research Council (SERC) working party did not "examine the case" for renewing the agreement with the South African Council for Scientific and Industrial Research (CSIR) over the South African Astronomical Observatory (SAAO) (*Nature* 23 September, p.291). According to Professor Wilcock presenting the report at Sussex, their remit was to assess future demand for astronomical facilities in South Africa.

So the working party did not ask whether the link with CSIR, responsible for military as well as civil research for the South African state, is academically acceptable; did not ask whether the benefits to South African astronomy under the current agreement have been available to South African people irrespective of race and colour; and did not ask whether we should guard against the transfer of technology and technical know-how that might assist the South African police and military, breaching at least in spirit the United Nations embargo. Nor were answers given at Sussex on how far the SAAO facilities could be available to individual astronomers without the formal agreement between the SERC and CSIR as state agencies being renewed. Perhaps because of the narrow remit, the working party did not look at scientific merit and take into account the comparatively low rating given to projects at SAAO by the scientific committees in recent years; rather, they advocated investment in additional instrumentation. Their promotional report in no way meets the need for a critical review of the South African collaboration, nor does it help astronomers in the United Kingdom to face up to their moral, political and scientific responsibilities.

MAX WALLIS

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Cancer trends

SIR — Gough and Gelband (*Nature* 15 April, p.598), commenting on the US cancer mortality graph reproduced in *Nature* on 4 March (p.4), suggest that the decline in "other" cancers since 1950, matched by a large increase in lung cancer to give a small overall rise, represents a real decrease in incidence not due simply to better diagnosis and treatment, attributing the fall in incidence of stomach cancers to changes in food preparation and preferences, and in cervical cancer to improved public health.

But there could be some direct connection between increasing incidence of lung cancer and decreases in other forms, if the population was genetically heterogeneous for susceptibility to cancer in general, with only the site of the growth determined by extrinsic carcinogens, in accordance with the "cancer-proneness" hypothesis¹. A cancer-prone smoker would then be at serious risk of lung cancer, whereas a cancer-prone non-smoker would be as likely to get it in the stomach or uterus, or wherever another carcinogen was effective. But anyone who is not cancer-prone will not develop cancer in the lungs or anywhere else, however much he smokes, though he may die of some other smoking-related disease: and, of course, 7 out of 8 of the heaviest smokers never do get lung cancer.

It follows that the more people take to

smoking the more of the cancer-prone will die of lung cancer, although total cancer incidence will remain much the same. Actually, total cancer mortality should rise a bit (as it has, by 7 per cent over 30 years), since lung cancer kills more quickly than most, with little hope of cure.

There is evidence² that where particular geographical areas show an excess of one form of cancer (for example, between town and country, or in different London boroughs³), this is usually balanced by lower mortality from other forms, as is so also with occupational and racial groups, and between the sexes. In England and Wales in 1980, for example, the Registrar General reported 27,755 men but only 8,773 women dying of respiratory neoplasms (ICD 160-165); but for all cancers (ICD 140-208) there were 68,882 male and 60,359 female deaths, since 12,167 women but hardly any men died of breast cancer, and with more female genital cancers as well. So it looks as though a lot of cancer-prone women, who would have got lung cancer had they smoked as much as men, are developing mammary and cervical cancers instead, incidentally with better prospects of survival. And these American trends are perhaps best explained by genetic cancer-proneness as well.

There is an important practical point here, since recent claims that individual susceptibility to cytotoxic and carcinogenic agents can be diagnosed by tissue culture techniques⁴ suggest that it may become possible to ascertain relative cancer-proneness in advance, and to advise accordingly on smoking habits and employment.

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Narrative style please

SIR — Sir Peter Medawar¹ comments on the "conventional" style (popular in the life sciences) as follows: "The layout of the text that has come to be regarded as conventional is that which perpetuates the illusion that scientific research is conducted by the inductive process. . . . A paper in the conventional layout may contain a section called 'Results' — a voluble pouring forth of factual information, usually with no connecting narrative to explain why one observation is made or one experiment done rather than another. Then follows a passage called 'Discussion' in which the author plays out the little *charade*. . ." (my emphasis).

In contradistinction, a recent paper in "continuous narrative" style by (for example) Charlton and Young² is not only of interest to fellow workers in their particular field but also to generalists, historians of chemistry and philosophers of science as a record of how their ideas developed during the research project. If one then takes into consideration the recent suggestion³ that even Francis Bacon was not an inductivist at heart, then the question might arise as to the possible inappropriateness of the "conventional" style in modern scientific journals.

Conventional methods of presentation have also been criticized by Norwich⁴, who states: "Experimental work is very often published in 'digested' form: raw data are not usually given. This may be due to author's preference, or due to editorial constraints, but in any case the lack of raw data often precludes the use of this paper in the evaluation of models," (my emphasis).

The American Chemical Society has arranged a system whereby an author deposits supplementary experimental details with the editor under a "masthead reference".

If this were allied to manuscripts being submitted in the "readable" style, then primary journals might become more useful records of scientific research.

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Perchlorate hazard

SIR — We reported last year¹ on an explosion which occurred in our laboratories during the preparation of cobalt (II) perchlorate. Subsequent investigations have revealed no evidence to suggest that the material as normally prepared, hexafluorocobalt (II) perchlorate, is unstable in the absence of oxidizable material. Experiments indicate that the accident was probably caused by heating the solid hexafluorocobalt compound to a temperature (~150 °C) at which partial loss of water of crystallization occurred with formation of a lower hydrate which is an endothermic compound capable of explosive decomposition. A fuller report has been published².

It seems that this type of hazard, which has not been previously recognized, may well exist with other metal perchlorates which normally exist in a stable hydrated state. Extreme caution is therefore urged in the handling of even pure metal perchlorates, to ensure that there is no risk of unintentional dehydration occurring. Even if this is done, it would be prudent not to subject perchlorates to mechanical shock unless the particular compounds involved are specifically known to be stable.

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Human conception

SIR — While entirely supporting Dr Edwards' work on *in vitro* fertilization and subsequent reimplantation of human embryos, and not wishing to become embroiled in the ethics of induced abortion, it seems to me that "the time . . . when human life begins" (*Nature* 7 October, p.475) must be at fertilization. Then, and only then, does the embryo contain the potential (that is, the genetic information) to develop into a whole human being.

J. R. BAKER

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NEWS AND VIEWS

Has the Ising lattice come of age?

This year's Nobel prize in physics (to Cornell's Dr Kenneth Wilson) should help to draw attention to a surprising field of enquiry, half way between statistical and quantum mechanics.

THIS WEEK'S announcement that Dr Kenneth Geddes Wilson of Cornell University is the sole recipient of this year's Nobel Prize in physics will (or should) direct attention to an imprudently neglected backwater of theoretical physics, the attempt going back to the 1930s to calculate the thermodynamic properties of cooperative systems, those in which the combined interaction between a collection of microscopic particles turns out to be greater than the sum of their separate interaction, in the condensation of gas into a liquid, for example. The underlying Ising problem can be simply put, and is now almost a textbook example (thanks to Onsager's analytical solution of it in 1944 (Onsager, L. *Phys. Rev.* **65**, 117)). Consider, as the textbooks say, a square lattice in a two-dimensional plane, let there be some two-valued physical entity at each vertex of the lattice, let immediately neighbouring entities interact with each other in some fashion and ignore all but next-nearest neighbour interactions. Primitively, such a system is a crude model of a variety of cooperative systems. If, for example, the entities at neighbouring vertices are tiny magnetic dipoles free to point in one direction or the opposite, their mutual interaction energy may be taken as negative if they point in the same direction and, without loss of generality, positive if they point in opposite directions, and the system as a whole is a model of a kind of ferromagnet. If, on the other hand, the two-valued entities at the vertices of the lattice are atoms of different kinds (say copper and zinc), the Ising lattice is potentially a model of a solid such as brass capable of an order-disorder transition. Most realistically, the two-dimensional Ising lattice can represent the condensation of a two-dimensional liquid on the surface of a solid absorbant with an underlying lattice structure. So will such a simple model, in which all but nearest neighbour interactions are neglected and in which there are only two, not three dimensions, mirror the real world, demonstrating phase transitions in particular? Onsager (who was awarded a Nobel Prize for something quite different, his penetrating work in irreversible thermodynamics) answered yes.

The Ising lattice problem has been a perpetual frustration ever since. In statistical mechanics, the prime objective is to calculate the partition function of the lattice, the quantity given by

$$Z(T) = \sum \exp(-E/kT)$$

where T is temperature, E is the energy of some configuration of two-valued spins on the vertices of the lattice, k is Boltzmann's constant and the sum is taken over all possible configurations of spin on the lattice as a whole. Onsager's trick was to reduce the partition function to a product. It remains the case, however, that the most natural extension of the Ising lattice problem, from two to three dimensions, has no analytical solution (but numerical calculations show that the partition function behaves as if there were an order-disorder transition at some critical temperature). Other such lattice problem have nevertheless revealed an unexpected interest of their own; Onsager himself, for example, showed that a low-temperature triangular lattice of interacting two-valued elements is thermodynamically identical with a high-temperature hexagonal lattice in which a hexagon of the second surrounds each vertex of the first. Since the early 1950s, the Ising lattice industry has modestly flourished, but analytical solutions of lattice problems remain few and far between. In this spirit a recent article reports the discovery of yet another solution of the Potts model — a simple extension of the Ising model in which the physical entities occupying the vertices of a two-dimensional

square lattice can "point", so to speak, in an arbitrary number of directions (Baxter, R.J. *Proc. R. Soc. A* **383**, 43–54; 1982).

Kenneth Wilson's achievement, now justly recognized, is to have cut through many of the mathematical obstacles to the analytical solutions of thermodynamic lattice problems by importing techniques developed in a quite different field — the apparently unrelated theory of particle fields, to be precise. Wilson's inspiration appears to have been two-fold. First, a decade ago, he seems to have been chiefly concerned to solve Ising lattice problems by a kind of bootstrap method. The argument might go like this. Take a square two-dimensional lattice, abolish half of the physical entities at the vertices and replace the others by square arrays of interacting entities. Geometrically, the new lattice is identical with the old, except that it has twice as many elements, so that to the extent that its thermodynamic properties are intensive (independent of extent) the calculations should yield the same results. So far, so good, but the transformed lattices cannot usually be calculated analytically. Wilson's second step therefore appears to have been to recognize the formal similarity between the calculation of a partition function (where energy is represented by a Hamiltonian defined as a function of configuration) and the calculation of quantities in particle field theory, where the underlying function (usually written in Lagrangian form) is a function of momenta, not of configuration coordinates. So why not use the investment over two decades by an army of field theorists to solve problems in statistical mechanics? That was the question Wilson seems to have asked himself. The result is that it is now possible to make approximate calculations of the properties of realistic models of cooperative systems near their critical points by importing results and techniques long-established in field theory. That, it turns out, is one side of this coin.

The other side of the coin is in its way even more intriguing, if less well understood. In field theory calculations, people are forever breaking their heads against the difficulty that supposedly physical quantities turn out to be, when calculated, literally infinite. Feynman was the first to show how these infinities can be eliminated from quantum electrodynamics in a self-consistent way, but mathematically they are no different from the ultraviolet catastrophe that plagued classical radiation theory until Planck abolished them by fiat. But why, Wilson seems to have argued in the mid-1970s, not avoid these troublesome infinities altogether by working with fields that have an underlying lattice structure, and which are then incapable of giving rise to ultraviolet (short wavelength) catastrophes? The outcome is the now-substantial intellectual industry called lattice-gauge theory, in which the inherent arbitrariness of an Ising lattice problem in thermodynamics (increasing lattice dimensions can always be compensated for by increasing nearest-neighbour interactions) mirrors the arbitrary gauge (up to a factor which is a complex number of unit modulus) of the electromagnetic field. A further important (in thermodynamics) by-product of that development is Wilson's demonstration of the value of the number of dimensions in which a model is constructed as a kind of physical variable in its own right. But it is possible that Wilson's finest achievement so far is to have persuaded one physicist working in statistical mechanics to confess, on Monday, that "we now talk regularly to the particle physicists". That is something really to be proud of.

Transposable elements, hybrid incompatibility and speciation

from David Ish-Horowicz

AN important milestone in the understanding of transposable genetic elements (transposons) in eukaryotic genomes has now been passed. Transposons, familiar enough in bacteria and first inferred in eukaryotes by classical genetic analysis in maize, have been identified in other eukaryotic genomes principally because their DNA sequences can crop up at different places. However, direct visualization of transposable events has not previously been possible because of their low frequency. Rubin and his colleagues^{1,2} have now characterized a class of transposons that can move at very high frequency and that appear responsible for a variety of genetic disturbances that are seen when wild and laboratory strains of *Drosophila* are interbred. Their direct demonstration of the transposition of these elements will further stimulate interest in eukaryotic transposons, provide a technique for the genetic manipulation of the *Drosophila* genome and provide further food for thought on questions such as the role of transposons in speciation.

When wild and laboratory stocks of *Drosophila* are interbred, the transposition of mobile genetic elements can cause a variety of genetic disturbances. The term 'hybrid dysgenesis'^{3,4} is used to describe symptoms which include enhanced mutation rates, hybrid sterility and the induction of chromosome rearrangements. The germ line of the hybrids only is affected; the first-generation hybrids appear morphologically normal but have reduced fertility, and their progeny show the other dysgenic traits. Hybrid dysgenesis is found only between wild males and laboratory female flies — not in the reciprocal cross — and appears to involve an interaction between the paternal genome and the maternal cytoplasm.

The paternal components can be mapped genetically and comprise a set of dispersed chromosomal elements. Because these elements can have different chromosomal locations, they were assumed to be mobile^{4,5}. Moreover, most of the mutations induced by dysgenesis are genetically unstable⁶, a characteristic of insertional mutations in prokaryotes. Two independent dysgenic systems (P-M and I-R) with similar genetic characteristics have been described, but which presumably result from different elements^{3,4}. The P-M system is the better characterized and a simple model suggests⁵ that wild stocks have a specific class of transposons (P elements) that induces

hybrid dysgenesis in eggs from susceptible females that lack P elements (M females).

Rubin *et al.*¹ have now shown that unstable mutations induced by P-M hybrid dysgenesis are indeed caused by the insertion of such a class of transposable (P) elements. Using gene cloning and Southern transfer-hybridization to analyse mutations in the *white* locus that had been induced by hybrid dysgenesis, they found that five out of seven mutations were caused by a set of insertions of differing lengths but homologous sequence. The mutations were stable in non-dysgenic crosses but tended to revert when exposed to P-M dysgenesis, with the loss of the inserted elements.

In a second paper², they analysed the distribution of P elements in various fly stocks and found that, as predicted, P elements are indeed present in P stocks (30–50 elements per haploid genome) and, with one exception, absent from M stocks. They also showed that dysgenesis causes high-frequency transposition of P elements into genetically marked M chromosomes, some of which then behave like P chromosomes; for example, they can cause sterility when crossed to M females. Bingham *et al.*² found a strong correlation between the number of acquired P elements and the ability of the M chromosome to induce dysgenic sterility. Finally, they showed that P elements are present at sites that are sensitive to chromosomal rearrangements induced by dysgenesis. This complements previous genetic work correlating rearrangement hot spots with P-element activity⁷.

Probably most, if not all, of the phenotypes associated with hybrid dysgenesis are caused by P-element transposition. Thus the chromosome rearrangements arise from chromosome breakage during transposition. Dysgenic sterility results from germ-cell death^{8,9}, presumably because of induced chromosome breakage and/or mutagenesis.

The molecular basis of the nuclear/cytoplasmic interactions in dysgenesis is less clear. Cytoplasm from P females appears to suppress P-element transposition, suggesting that the P-elements encode repressors of transposition (or inducers of host-encoded repressors). But this is an oversimplification because resistance to dysgenesis shows partial maternal inheritance^{10,11}, so it may be that (although experimental support is lacking) cytoplasmic resistance is determined by P elements that can undergo limited extrachromosomal inheritance.

What about the M stock that contains P elements? Bingham *et al.*² suggest that some elements are defective for some P

functions and that this exceptional M stock contains only inactive P elements. This is supported by the great variation of size of chromosomal P elements² and the variability of the P insertions in the dysgenic *white* mutations. Moreover, although the P elements in the *white* mutations are mobilized by dysgenesis, they cannot mobilize themselves, nor do they confer cytoplasmic resistance to dysgenesis. The existence of partially defective elements which can be transposed but which are incapable of catalysing transposition is reminiscent of the *Ds* element in maize^{12,13}.

So far, I have suggested that P-M dysgenesis acts specifically to mobilize P elements. However, two of the dysgenically induced *white* mutations were due to the insertion of the transposable element *copia*, which is not related to the P element. It is found in both wild and laboratory *D. melanogaster* stocks and in a wide variety of other *Drosophila* species^{14–16}, so its mobilization by P-M dysgenesis is somewhat unexpected. If other elements are mobilized by dysgenesis (this is not yet known), the P-encoded transposase must show relaxed specificity. But, in fact, in its choosing of chromosomal insertion sites for P elements it seems rather selective. Thus three out of the four dysgenic *white* mutations analysed by cloning are due to insertions at the same site ($\pm$ 50 base pairs), and dysgenic mutagenesis shows clear locus specificity^{6,17}. An alternative explanation could be that the *copia* transposition is due to the induction of its own transposition machinery by the 'trauma' of dysgenesis, along the lines of SOS induction of phage λ excision.

An apparent discrepancy exists between the rates of insertion and excision induced by dysgenesis. Bingham *et al.*² found an average of five newly inserted P elements per X chromosome within two generations,

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which is more than would be expected even if all P elements were mobilized during dysgenesis. (The X chromosome comprises about one-fifth of the genome. A specific X would acquire one-tenth of the P elements if they were redistributed randomly. Thus, on average, the mean number of elements acquired by an X chromosome from a P-M hybrid containing 30 P elements would be 3.) However, P elements excise at a much lower frequency, typically 10^{-2} – 10^{-3} per generation^{2,6}, so all the newly inserted P elements cannot have derived from excised elements. Similarly, there is a disparity between the transposition and excision rates of *copia* during dysgenesis. The *white* mutations caused by *copia* insertion appear relatively stable during dysgenesis¹ so that excised elements are unlikely sources of the insertional elements. There are two obvious ways of explaining these results. The first is that transposition is replicative (as for prokaryotic elements^{18,19}), such that the original copy is retained while a novel insertion is generated. This leaves open the mechanism of excision which, being induced by dysgenesis, presumably requires P element-encoded functions (in contrast to the precise excision of prokaryotic transposons which appears to be host-encoded²⁰). The second possibility is the insertion of extrachromosomal

elements — extrachromosomal *copia* elements have been identified in *Drosophila* tissue-culture cells²¹. As mentioned above, extrachromosomal P elements would also account for some of the maternal inheritance of cytoplasmic susceptibility to dysgenesis.

We can now expect a detailed molecular study of the P element and the mechanism of its transposition. Complementary studies on the I-R dysgenic system will clarify differences with P-M dysgenesis⁴. We are left with the question of the origin of hybrid dysgenesis, and its relevance to other biological systems. It has been suggested that dysgenesis between *D. melanogaster* populations may be a step towards speciation^{8,9}, and hence that hybrid sterility between species may also be due, in part, to mobile elements^{2,5}. Different elements arising in isolated populations could lead to hybrid sterility and reproductive isolation, and ultimately to speciation. Clearly this notion is highly speculative, but at least it is based on clear experimental data. There are reports of interbreeding sterility resembling hybrid dysgenesis in other insect species, but exact analogies cannot be made for lack of detailed genetic characterization. Whether speciation is usually due to transposable elements awaits studies on species-specific transposable elements. □

Antarctic sulphate budget

from Robert J. Delmas

ANTARCTICA represents a vast island remote from the other continents of the Southern Hemisphere. Most of the continent is covered by several kilometres of ice and the total ice-free area is very small. On the high central plateau the air is the cleanest in the world and the impurity content of the snow, less than about $0.3 \mu\text{g g}^{-1}$, bears witness to the fact that very few substances are transported to these regions. Mineral acids, especially sulphuric acid, seem to dominate the bulk of deposited trace substances¹ in central Antarctica.

In this issue of *Nature* (p. 710), Radke reports measurements which suggest that Mt Erebus, the only significant active volcano on the continent, could be an important source of aerosols, and particularly of sulphur compounds, for the entire Antarctic atmosphere. At other latitudes its contribution ($\sim 4 \times 10^7 \text{ kg yr}^{-1}$ of sulphate) would be considered insignificant as an aerosol load on a continental scale. However, in the clear air in Antarctica this source could have a dramatic effect on atmospheric chemistry.

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In the light of Radke's new values, must the Antarctic sulphate budget be revised?

The impact of such a volcanic input of sulphur dioxide must be examined in relation to the present-day sulphur cycle in Antarctica. Analysis of chemical impurities in snow has given qualitative and quantitative information about the various sources of the sulphate in Antarctica. Concentrations in snow are generally in the range 0.05 – $0.15 \mu\text{g g}^{-1}$ of sulphate, and sea-salt sulphate (deduced from the sodium content of snow) has been found to be significant (about 10 per cent of the total) in central areas^{1,2}.

As in other parts of the world, the

Antarctic sulphate budget contains what is commonly referred to as 'excess sulphate'. In the Antarctic this fraction is most generally in the form of sulphuric acid^{1,3} with no significant amounts of ammonium being detected⁴. The excess sulphate may come from the extensive oceans surrounding the Antarctic continent, which are considered to be sources of reduced gaseous sulphur compounds on a global scale. Marine biogenic activity is responsible for the formation of these not yet fully identified compounds, which may be dimethylsulphide⁵ and COS (ref. 6). The particle sizes of gas-derived, or secondary, aerosols is very small (0.05 – $0.2 \mu\text{m}$) and characteristic. Over the ice sheet Shaw⁷ found evidence of an ultrafine aerosol, probably originating from sub-Antarctic areas. The aerosol has a much longer residence time (more than a month) than at other latitudes, largely due to the low precipitation, on average about $12 \text{ g cm}^{-2} \text{ yr}^{-1}$, in Antarctica.

A third source contributing to the sulphate content of snow has been clearly identified⁸ in the annual snow layers corresponding to the two major eruptions of Krakatoa (1883) and Agung (1963). This sporadic volcanic contribution, which has been estimated to account for possibly a third of the total sulphate deposition, is most probably related to the sulphate from the stratospheric Junge layer, for which sulphate content is directly linked to hemispheric explosive volcanism. The contribution of local (Antarctic) volcanoes has previously been estimated to be negligible⁸ in view of the sulphur emissions reported previously⁹ for Mt Erebus.

So what impact does Erebus have on the Antarctic sulphate budget? The table shows the flux of sulphur compounds passing over the ice sheet, calculated roughly from available data using a simple box model sufficient to give an order of magnitude and a basis for comparison. The sulphur emitted from Mt Erebus (mainly as sulphur dioxide) enters the Antarctic atmosphere together with the sulphur dioxide and excess sulphate already present in the air masses. It appears that the sulphur from Erebus ($4.2 \times 10^7 \text{ kg yr}^{-1}$) represents only a small part of the total sulphur ($1.5 \times 10^9 \text{ kg yr}^{-1}$) transported by the air masses over the ice sheet. It can also be estimated that only a small part (about 10 per cent or $13 \times 10^7 \text{ kg yr}^{-1}$) of the total is

Selected data for the sulphur cycle in Antarctica

	Amount ($\times 10^9$) kg per year		
	SO ₂ (as SO ₄)	SO ₄	Total (as SO ₄)
Deposition in Antarctica	0.05 (a)	0.13 (b)	0.18
Input flux to Antarctica	0.5 (c)	1 (c)	1.5
Emitted from Mt Erebus	0.04 (d)	0.002 (d)	0.042

The Antarctic atmosphere is represented by a rectangular box ($3,700 \times 3,700 \times 5 \text{ km}$; total area $14 \times 10^6 \text{ km}^2$). Typical average concentrations in the air are: SO₂, $0.1 \mu\text{g m}^{-3}$ and SO₄, $0.3 \mu\text{g m}^{-3}$. Average wind speed, 5 m s^{-1} ; deposition velocity of SO₂ on snow, 0.08 cm s^{-1} .

(a) Estimated dry deposition (concentration in the air $\times$ deposition velocity), assuming uniform concentration in the Antarctic air⁸. (b) From measurements in Antarctic snow¹⁰. (c) Calculated by using a simple box model (see text). (d) From Radke (*Nature*, this issue, p. 710).

deposited in the Antarctic. This percentage is probably true also for the contribution from Erebus, so that the amount of excess sulphate due to this source, which is apparently significant when compared with the sulphate deposited in Antarctic snow, may in fact have only a small influence on the Antarctic sulphate budget.

The residence time of sulphate in the Antarctic atmosphere can be calculated from the table to be about 50 days which is in good agreement with Shaw's estimate⁷. Assuming a mean wind speed of 5 m s⁻¹ for the air masses it is possible to estimate that the sulphur emitted from Erebus may be transported over very large distances (13,000 km per month), and that it probably falls out over the sub-Antarctic

ocean surfaces, where aerosol removal is very rapid and efficient.

Clearly further studies are still needed for a complete understanding of the sulphur cycle in Antarctica, particularly on the gas-to-particle conversion of sulphur in the polar atmosphere. []

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Promiscuous DNA—chloroplast genes inside plant mitochondria

from John Ellis

THE cells of photosynthetic eukaryotes are genetically the most complex that have been discovered, since their development requires the interaction of three genetic systems, in the nucleus, the mitochondrion and the chloroplast. These systems are usually considered to be discrete and separate in both their specific functions and their nucleotide sequences. The article by Stern and Lonsdale in this edition of *Nature* (p.698) overturns this simple view by its demonstration that the mitochondrial DNA of maize plants contains a nucleotide sequence homologous to part of the chloroplast DNA of the same and other species.

Most polypeptides found in the bioenergetic organelles are encoded and synthesized within the organelles themselves. Current thinking about the interaction of the three genetic systems stresses the role of the transport into chloroplasts and mitochondria of proteins made on cytoplasmic ribosomes from nuclear-encoded messenger RNAs¹⁻³, which can be readily demonstrated in isolated subcellular systems and which is coming increasingly under study because it differs in mechanistic detail from the secretion of proteins across the endoplasmic reticulum^{4,5}. But in spite of speculation⁶, there is no good evidence that messenger RNAs traverse the chloroplast or mitochondrial envelope in either direction, or that proteins made inside these organelles function outside them.

The discovery by Stern and Lonsdale that mitochondrial and chloroplast DNAs share a common sequence opens up a new way of thinking about the evolution and maintenance of these genomes. Movement of DNA within the nuclear genome was

first demonstrated by B. McClintock during genetic experiments with maize plants in the 1940s, and is a well established general phenomenon⁷. We must now also consider the possibility of DNA movement between the organelles of eukaryotic cells. Since such movement diminishes the separate identity of each genome, I propose the term 'intracellular promiscuity' to describe this phenomenon.

Promiscuous DNA is defined as a nucleotide sequence which occurs in more than one of the three membrane-bound organellar genetic systems of eukaryotic cells, and should be distinguished from the DNA of promiscuous plasmids, which can be transferred to a wide range of prokaryotic cells.

In the work they have now described, Stern and Lonsdale prepared a series of cosmid clones containing a set of overlapping sequences of mitochondrial DNA isolated from maize plants. The inserted DNA has been shown by hybridization and restriction endonuclease analysis to contain a 12 kilobase region homologous to a region of maize chloroplast DNA which encodes the chloroplast 16S ribosomal RNA and several chloroplast tRNAs. A possible criticism of these experiments is that the preparations of mitochondrial DNA used were contaminated with chloroplast DNA. Most practitioners in this field acknowledge that it is difficult, if not impossible, completely to prevent cross-contamination between the organellar nucleic acids of plant cells. Stern and

Lonsdale used differential centrifugation rather than gradient centrifugation to prepare their mitochondria for the sake of speed and thus to minimize the degradation of mitochondrial DNA, but it is not surprising that they did detect some contamination of mitochondrial nucleic acid by chloroplast nucleic acid.

Fortunately, cross-contamination can be ruled out as an explanation of their results. The cosmid clones contain border regions, or sequences, where the restriction pattern characteristic of mitochondrial DNA joins sequences with the restriction pattern characteristic of chloroplast DNA. The critical observation is that the same border regions are present in uncloned mitochondrial DNA but are not present in chloroplast DNA. If the border regions in cloned DNA were chimaeras of chloroplast and mitochondrial DNA perhaps formed during cloning, it is exceedingly improbable that they should also occur in uncloned DNA.

Nothing is known about the possible function or origin of the promiscuous sequence in maize mitochondrial DNA, but it is interesting to speculate in terms of the endosymbiont hypothesis for the origin of eukaryotic cells⁸. This hypothesis supposes that chloroplasts and mitochondria originated from free-living prokaryotic cells which entered the eukaryotic progenitor cell and evolved into a stable relationship with it. To account for the current dominance of the nucleus over the chloroplasts and mitochondria, this hypothesis must further suppose that a transfer of DNA from these organelles to the nucleus has occurred. A recent report in *Nature* of the presence in the mitochondrial DNA of *Neurospora* of a gene coding for a protein homologous with a nuclear-encoded protein⁹ suggests that gene duplication, followed by transfer of one of the genes to the nucleus, may have been responsible. The results of Stern and Lonsdale add to this process the transfer of DNA from chloroplasts to mitochondria.

How could such transfers of DNA have taken place, and are they still happening? Several possibilities can be entertained. Chloroplasts and mitochondria may occasionally lyse and release their DNA into the cytoplasm, which could enter other organelles and become partially integrated into their genomes. (It is well established that DNA injected into the cytoplasm of animal cells can be integrated into the nucleus and expressed¹⁰.) A more direct transfer of DNA may occur if different organelles occasionally fuse together, and indeed, outer membrane continuities between mitochondria and chloroplasts have been seen in electron micrographs of fern prothallia¹¹, while direct evidence of fusion in living mesophyll cells was claimed many years ago by Wildman in his famous film¹².

The most attractive possibility, however, is that plasmid or virus-like vector DNA has carried sequences between organelles.

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Both linear transposon-like elements and small circular DNA molecules have been found in maize mitochondria¹³, and Stern and Lonsdale point out that the identification of a vector transporting DNA between organelles could be important in realizing the potential for the genetic manipulation of plants¹⁴.

Many questions are raised by the discovery of promiscuous DNA. Is it significant that the sequences now identified are confined to non-protein-encoding genes? Are the chloroplast genes inside mitochondria transcribed? Does chloroplast DNA contain mitochondrial genes? Are chloroplast genes also present in the nucleus? Is promiscuity a rare evolutionary event that confers selective value on the hybrid molecule or is DNA continually shuttling between organelles? A hint that the promiscuous sequence in maize may be essential for normal plant development is provided by the finding of Stern and Lonsdale that this sequence is altered in mitochondrial DNA, but not chloroplast DNA, of maize plants which are male-sterile.

The organelle field is currently submerged in a fashionable ocean of sequencing for its own sake; it is to be hoped that this report will stimulate researchers to pursue the more interesting problem of the extent and significance of promiscuous DNA. □

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Sub-oceanic heat-flow from above

from Peter J. Smith

THE obvious and usual way of determining the flow of heat through the oceanic crust is to drop a thermal probe into the sediment on the ocean floor. The temperature gradient thus obtained is then combined with the thermal conductivity of the sediment, independently estimated or (preferably) measured, to calculate the rate of heat flow at the site in question. It is a simple method in principle but time-consuming in practice, requiring access, albeit by remote control, to the ocean floor. However, Yamano *et al.* (*Geology* 10, 339; 1982) have now devised a remarkable procedure for determining oceanic heat flow without having to go near the ocean floor and without even having to measure temperature. All that are required are seismic measures of depth and velocity, which in many cases have probably been obtained already for other purposes.

What makes the method possible is the existence in some parts of the oceanic crust of gas hydrates — crystalline ice-like substances formed by the combination of water with gases such as methane (chiefly) and carbon dioxide. If there is sufficient gas around, hydrates will form where the temperature is low enough; and in that event there must then be a depth below which the temperature is too high to allow hydrates to exist. In other words, there will be a phase boundary separating the gas hydrates and sediments above from the non-hydrated sediments below. A phase

boundary represents a density discontinuity and hence a seismic velocity discontinuity, which means that a gas hydrate phase boundary will often show up as a prominent reflector in seismic profiling. Once detected in this way, it is then a simple method to determine the boundary's depth.

From that depth and the seismic P-wave velocity in the surrounding sediments (obtainable from multichannel seismic records) everything else follows. The P-wave velocity gives the density, for P-wave velocity-density relationships for marine sediments have been compiled by Hamilton (*Acoust. Soc. Am. J.* 63, 366; 1978). The density and boundary depth (which equals the height of overlying sediment column) may then be combined to calculate the pressure at the phase boundary. But the phase (pressure-temperature) diagram for gas hydrates is now reasonably well known, and so pressure leads straight to temperature. The temperature gradient between the phase boundary and the surface of the oceanic crust may then be calculated using the bottom-water temperature, obtainable from physical oceanographic tables. Finally, the P-wave velocity also gives the thermal conductivity, for Horai (*Init. Rep. DSDP* 60, US

Govt Printing Office, Washington DC; 1982) has recently derived P-wave velocity-thermal conductivity relationships for marine sediments. In short, all the required information is available to determine heat flow.

But can the principle be put into practice with any confidence? Not only are Horai's P-wave velocity-thermal conductivity data fairly widely scattered about the best-fit curve, but they were obtained from boreholes drilled on only one DSDP voyage, which raises the question of the extent to which the best-fit curve is universally applicable. Likewise, there may well be doubt about whether Hamilton's general P-wave velocity-density relationship will hold in all individual circumstances. There is also some evidence (Stoll and Bryan *J. geophys. Res.* 84, 1629; 1979) that the very presence of gas hydrates increases the P-wave velocity and decreases the thermal conductivity with respect to values in normal sediments, although these effects appear to be small as long as the gas hydrate layers are not very thick. Finally, there is bound to be some error introduced by uncertainties in ocean floor temperature and the gas hydrate phase diagram. Nevertheless, Yamano and his colleagues estimate that the maximum error in their derived heat flow values is no more than 25 per cent.

The better test of validity, however, is perhaps not the theoretical estimation of error but comparison with conventional heat flow measurements. Yamano and his colleagues have therefore tried out their method in three areas — across the landward slope of the Nankai trough (off Japan), around central America and on the Blake outer ridge. Twelve heat flow values thus derived from a seismic profile across the Nankai trough slope range from 1.20 to 1.91 HFU ($\mu\text{cal cm}^{-2} \text{ s}^{-1}$), which is almost the same range as that covered by values obtained by conventional methods. This particular test is not, in fact, as convincing as it might appear, for there are very few conventional heat flow values available for comparison. However, the Nankai trough is unusual in that on its floor the heat flow as measured conventionally rises to above 3 HFU. What is impressive is that the range of values obtained by the Yamano method on the trough slope excludes the much higher values characteristic of the nearby trough floor. Moreover, in the case of another slope profile, one end of which actually projects into the trough floor area, higher (up to 2.6 HFU) derived heat flow values are obtained at that end. The method thus appears to be able to resolve spatial variations in heat flow quite well.

Additional confidence in the validity of the method is also provided by the other test regions. On the Blake outer ridge, for example, conventional heat flow values range from 0.91 to 1.91 HFU and Yamano values from 1.00 to 1.15 HFU. Similarly, in the eastern Pacific off central America, values derived using the Yamano pro-

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cedure range from 0.69 to 1.15 HFU whereas conventional values fall in the range 0.60–1.29 HFU.

The technique developed by Yamano and his colleagues, which is in any case limited to regions in which gas hydrates occur, has not yet been widely tested, so it is not possible to say whether or not it will prove to have wide application. Clearly, individual Yamano heat flow values will

generally be less accurate than conventional values; but on the other hand, by taking temperature gradients over greater depths and by using more generalized thermal conductivity and temperature data, Yamano values will be the more integrative, representing heat flow throughout a larger region. In any case, the accuracy of the procedure could well improve with time, given better-constrained input data.

(together with the radio) the timing of particle acceleration during flare onset.

Activity thought to be 'Sun-like' is associated with stars quite different from that of the red dwarfs, but the similarity may be misleading. T Tauri stars, such as the RS CVn and W UMa stars, are close binary systems, but in the opinion of this reporter, observations of these relatively unusual star systems must be interpreted with great caution; in close binaries, tidal forces are likely significantly to influence the interior flow structure, so that the nonlinear dynamics governing magnetic field generation is virtually certain to be different from the Sun's.

As it turns out, progress in the theoretical area, while rapid, has not kept pace with the observations. E. Priest (St Andrews University) described current understanding of magnetic instabilities in plasmas in which the magnetic field dominates the dynamics (as expected in stellar coronae). Although linear theory is well understood, nonlinear theories provoke the crucial question of which instability ultimately dominates and, in particular, whether resistive instabilities ultimately determine the evolution of magnetic coronal structures (such as the 'loops' seen in solar X-ray images).

Magnetic field-related dissipation and plasma-heating associated with such magnetohydrodynamic instabilities are, of course, essential to the flaring process as now understood. The complexity of the global problem of flares has recently revived interest in 'electric circuit' analogies of the flaring atmosphere (D. Spicer, ETH), as originally propounded by Alfvén and others in, for example, terrestrial magnetosphere studies. Such analogies focus attention on the fact that stellar surface phenomena are strongly coupled to subphotospheric processes, and that a solution of the 'flare problem' will ultimately have to take account of the plasma-magnetic field interactions at these photospheric (or deeper) layers that 'drive' the instabilities above the stellar surface and which are themselves still poorly understood.

This is especially true of the instabilities that must give rise to the highly inhomogeneous spatial structure of magnetic fields (including spots) at the surfaces of stars such as the Sun (D. Mullan, Bartol). In red-dwarf stars in general, the theoretical difficulties well known in the solar case are compounded by the circumstance (U. Bohn, University of Würzburg) that the structure of the outer convective zone (including such basic parameters as the opacity as a function of depth) is very poorly known. Furthermore, recent work in nonlinear dynamics has shown that the behaviour of nonlinear systems (of which stellar flares and the

Activity in red-dwarf stars

from Robert Rosner

MANY red-dwarf stars exhibit rotational light-curve modulation (the BY Draconis phenomenon) and flaring behaviour in the optical and radio regions of the spectrum (the UV Ceti phenomenon). Within the past decade, improved detectors, satellites and radio interferometry have led to a revolution in our understanding of phenomena occurring at the surfaces of these low-mass stars, as a recent colloquium* on this subject, held at Catania, Italy, made plain.

The central question is the root cause of the activity on the surface of such stars and, subordinately, to what extent the Sun is an analogue. These questions direct attention to the problem of understanding why stars differing widely in mass (from roughly twice solar to the least massive) are enveloped by tenuous plasma (the chromosphere and corona) whose temperature is substantially above that of the stellar surface. The remarkable observations of such hot plasmas by the International Ultraviolet Explorer (IUE) and the Einstein Observatory, which allow exploration of coronal phenomena over a wide range of stellar parameters, may shed light on mechanisms for the generation of magnetic fields in such stars, which have hitherto been based only on solar observations. The prospects for a better understanding of the workings of our Sun has, needless to say, not escaped attention.

Red-dwarf surface activity is probably an extreme version of the more familiar activity on the surface of the Sun. Detailed modelling of observations of quiescent emission in the UV and optical (J. Linsky, JILA), X-ray (L. Golub, SAO; H. Johnson, Lockheed; R. Stern, JPL) and, most recently, radio (D. Gibson, University of New Mexico) spectra suggest that the quiescent atmosphere overlying the cool photospheres of these stars resembles the portions of the solar atmosphere near sunspots (the 'plage' and 'active' regions). Moreover, new techniques and instruments used in ground-based studies yield novel (and strong) constraints on photospheric

models of the surface 'spot' activity.

Thus, S. Vogt (Lick Observatory) showed recent results of a doppler imaging of stellar surfaces based on the kind of expectation that spectra of surface features smaller than the projected stellar disk should be less broadened by rotation than the spectrum of the stellar disk as a whole; here one can track (using the Coudé spectrograph on the 3 m telescope) the wavelength displacement of narrow spectral features associated with stellar surface inhomogeneities as they rotate (with the star's surface) through the field-of-view, and thereby constrain the geometry and location of these inhomogeneities. These results support and extend earlier work showing that the periodic optical light modulation of red dwarfs can be understood as an extreme manifestation of solar-like 'star spots' (reviewed by Vogt), and complement the classical optical monitoring of red-dwarf activity by groups such as that at the University of Catania/Catania Observatory.

Similarly, red-dwarf transients (S.P. Worden, UCLA) are more remarkable for their resemblance to solar flares than for their differences therefrom. Indeed, the resemblance has become the more striking as instrumentation and measurement techniques have improved. The classical optical photometry of stellar flares (P. Byrne, Armagh) has been supplemented by observations ranging from the radio to X-ray regions of the spectrum. The Einstein observations (B. Haisch, Lockheed) in particular suggest that earlier inferences that stellar flares are X-ray deficient were due to instrumental spectral bias, and that red-dwarf flare spectra in the optical and UV (M. Giampapa, CfA) and X-ray regions have time scales and physical parameters closely resembling those of solar flares of comparable energy. But recent solar observations of flares in the keV region by SMM (G. Simnett, University of Birmingham) and HINOTORI (K. Tanaka, University of Tokyo) convinced this reporter that hard X-ray observations are a crucial missing element in the stellar area, probing

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processes governing stellar magnetic field generation are examples) can be drastically different from that expected on the basis of linear theory (N.O. Weiss, University of Cambridge). The particular nonlinear example discussed by Weiss — the stellar magnetic dynamo — has an extremely rich repertoire of possible solutions which are inaccessible to classical linear theory. As emphasized by G. Belvedere (Catania), there is considerable doubt whether the variation of stellar activity with spectral type (or stellar mass) and stellar rotation rate can be understood on the basis of textbook linear dynamo theory.

Where are current research trends leading us? On the theoretical side, the immediate future will probably see significant effort in understanding the departure from the linear regime in plasma and MHD instabilities, as well as in the magnetic flux generation problem; extant work already suggests that our present intuition is a poor guide in predicting the nonlinear behaviour of magnetized

plasmas. As pointed out by G.S. Vaiana (University of Palermo), the thrust of new instrumentation will continue to lie in the uncompromising quest for higher sensitivity (which allows for the possibility of high time and spectral resolution); significant advances in the relatively unexplored areas of high-spatial resolution radio astronomy and of XUV astronomy is particularly to be expected. It is the joining of theory and observations that may, however, be the most difficult. Not only is such a connection difficult to establish but, as exemplified by D. Soderblom's (CfA) study of calcium line emission properties of red-dwarf stars, exploration of stellar activity as a function of parameters defining the physical state of a star is subject to all the classical problems of observational stellar astronomy — including selection effects based on incomplete samples. It is the passage from anecdotal to systematic exploration which may be the most important new step in the study of stellar activity. □

that Edwards' argument is false, but the error is a subtle one related to a long-standing difficulty with the Principle of Least Action for continuous systems. At least four papers refuting Edwards' claim have recently been published⁴⁻⁷.

Edwards' argument was based on the classical action integral for charged particles in an electromagnetic field

$$I = \int \frac{F_{\alpha\beta} F^{\alpha\beta}}{4\mu_0} d^4x + \int \int \sum_i q_i \delta^4(x^\mu - x_i^\mu) \mu_{i\alpha} A^\alpha d\tau_i d^4x + \int \sum_i m_i c (u_{i\alpha} u_i^\alpha)^{1/2} d\tau_i \quad (4)$$

in which x_i^μ are the four-coordinates of a particle of charge q_i and mass m_i . The four-velocities are $u_i^\alpha \equiv dx_i^\alpha/d\tau_i$ and the field tensor is $F_{\alpha\beta} \equiv \partial_\alpha A_\beta - \partial_\beta A_\alpha$. The first term in equation (4) represents the free electromagnetic field and the third the free particles. The second term, in which the δ function connects the variables x^μ of the electromagnetic field with the position of the particles, represents the interaction between field and particles.

By the principle of least action, I must be stationary for arbitrary variations of the potentials A_α and of the particle paths x_i^μ . Variation with respect to the potentials yields Maxwell's equations for the electromagnetic field. Variation with respect to the particle paths would yield the conventional equations of motion for the charged particles, but Edwards does not introduce this second variation. Instead he expresses the action in terms of the current density j_α which he then treats as a field variable similar to A_α . The condition that I be stationary with respect to arbitrary variations of j_α leads to the equation

$$\mathbf{j} + \frac{ne^2}{mc^2} \mathbf{A} = 0 \quad (5)$$

which, bearing in mind that the field has arbitrary gauge, is equivalent to the London equation (2). Thus, by changing from a lagrangian description in terms of particle coordinates $x_i(t)$ to an eulerian description in terms of current $\mathbf{j}(\mathbf{x}, t)$, the equation of motion is replaced by the London equation.

Clearly something is amiss. A simple analogy illustrates what has happened. Consider a single non-relativistic particle in a static magnetic field. Its action integral is

$$I = \int \left(\frac{1}{2} m \dot{\mathbf{x}}^2 + \frac{e}{c} \dot{\mathbf{x}} \cdot \mathbf{A}(\mathbf{x}) \right) dt \quad (6)$$

and the principle of least action for a variation $\delta \mathbf{x}(t)$ in the path leads to the standard equation of motion. However, if one expresses the action in terms of velocity as

$$I = \int \left(\frac{1}{2} m \mathbf{v}^2 + \frac{e}{c} \mathbf{v} \cdot \mathbf{A} \right) dt \quad (7)$$

A classical derivation of the Meissner effect?

from J. B. Taylor

A SURPRISING controversy has surfaced in the usually sedate pages of *Physical Review Letters*, one of the most distinguished physics journals in the world. The argument centres around an assertion by W. Farrell Edwards of Utah State University that the familiar Meissner effect in superconductors may be explicable within the context of classical mechanics, rather than being a quantum phenomenon as it is usually supposed to be.

The Meissner effect is one of the crucial distinctions between a perfect conductor—one whose resistivity is zero—and a superconductor. In a perfect conductor any magnetic field $\mathbf{B}$ is 'frozen-in' so that $\partial \mathbf{B} / \partial t = 0$ except at the surface, but $\mathbf{B}$ itself may have any value. In a superconductor the field is expelled and $\mathbf{B} = 0$ except at the surface.

For a perfect conductor, in which electrons are supposed to be freely accelerated, the rate of change of current is related to the electric field by $\partial \mathbf{j} / \partial t = (ne^2/m) \mathbf{E}$ where n , e and m are the electron density, charge and mass. But $\mathbf{E} = -\partial \mathbf{A} / \partial t$, where $\mathbf{A}$ is the electromagnetic potential, so that

$$\frac{\partial}{\partial t} \left(\mathbf{j} + \frac{ne^2}{mc} \mathbf{A} \right) = 0 \quad (1)$$

Almost 50 years ago London and London¹ suggested that for superconductors this equation should be supplemented by

$$\nabla \times \left(\mathbf{j} + \frac{ne^2}{mc} \mathbf{A} \right) = 0 \quad (2)$$

Then, using Maxwell's equations, the magnetic field satisfies

$$\nabla^2 \mathbf{B} - \frac{4\pi ne^2}{mc^2} \mathbf{B} = 0 \quad (3)$$

and so decays exponentially into the superconductor, over a distance $(mc^2/4\pi ne^2)^{1/2}$, as required by the Meissner effect.

When equation (2) was introduced there was no attempt to derive it from a fundamental theory of superconductivity, for none existed. Nevertheless, even though it does not contain Planck's constant, it was always recognized that its justification lay in quantum mechanics and was associated with 'rigidity' of the wave-function for the superconducting electrons. This has, of course, been confirmed in the modern (Bardeen, Cooper and Schrieffer) theory of superconductivity. There was therefore some surprise when a paper appeared last year in *Physical Review Letters*² claiming to give a purely classical derivation of the London equation and so of the Meissner effect. Indeed, so remarkable was this claim that it reached the august columns³ of *The Times*. It is now generally agreed

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then variation with respect to $\mathbf{v}$ leads to $(m\mathbf{v} + e\mathbf{A}/c) = 0$, equivalent to the London equation, which is plainly not the correct equation of motion.

A similar difficulty with action principles in eulerian variables is well known in fluid dynamics⁸. There the kinetic and potential energies are both naturally expressed in eulerian variables and (ignoring entropy changes for simplicity) the action is

$$I = \int \int (\frac{1}{2}\rho\mathbf{v}^2 - U(\rho)) d^3x dt \quad (8)$$

where ρ is the density and $U(\rho)$ the internal energy of the fluid. The principle of least action for an independent variation $\delta\mathbf{v}$ would lead to the equation of motion $\mathbf{v} = 0$. But obviously one should not treat $\mathbf{v}$ and ρ as independent, for they are linked by the equation of continuity

$$\frac{\partial\rho}{\partial t} + \nabla \cdot (\rho\mathbf{v}) = 0 \quad (9)$$

However, when this is introduced as a constraint, through an undetermined multiplier λ one obtains $\mathbf{v} = \nabla\lambda(\mathbf{x}, t)$ which is still too restrictive, since it permits only irrotational flow. Hence the problem with the eulerian action principle is not simply that the variables are interdependent but involves something more subtle.

The essential point appears to be⁹ that in using the principle of least action to calculate equations of motion, the displacements $\delta\mathbf{x}$ must vanish at the initial and final times. This is more stringent than the requirement that the eulerian variables $\delta\rho$, $\delta\mathbf{v}$ should vanish for that does not exclude an interchange of indistinguishable fluid elements. To allow for this, one must impose a further constraint on the velocity:

$$\frac{\partial\alpha}{\partial t} + \mathbf{v} \cdot \nabla\alpha = 0 \quad (10)$$

for an arbitrary function $\alpha(\mathbf{x}, t)$. This constraint, first introduced by Lin¹⁰, has the effect of labelling each fluid element and is necessary and sufficient to make the principle of least action valid in eulerian variables.

It is clear that an equivalent of the Lin constraint should be incorporated into Edwards' argument. When this is done one obtains equations similar to equation (1) describing a perfect conductor, not the London equation for a superconductor.

In his response¹¹, Edwards appears to accept the force of this criticism but maintains that there may nevertheless be some classical systems for which it is correct to omit the Lin constraint. (Exactly why omitting it produces the correct result for superconducting electrons is not clear, but is presumably related to the impossi-

bility of following exact trajectories in quantum systems.) Edwards suggests that high-temperature plasmas may be a case in point and he refers to the existence of filamentary magnetic structures in plasmas (as occur in type II superconductors). There are, however, several possible explanations for these structures.

The reference to the behaviour of magnetic fields in plasmas gives a final intriguing twist to the controversy. Some years ago I proposed a theory of relaxation of turbulent plasmas¹², for which there is now considerable experimental support¹³. In that theory, the plasma relaxes to a final state satisfying $\nabla^2\mathbf{B} + \mu^2\mathbf{B} = 0$. This has a superficial resemblance to equation (3) but in this case μ^2 is a macroscopic parameter, related to the size of the system, and its sign is the opposite of that needed to describe the Meissner effect! □

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100 years ago

NEW OR RARE ANIMALS IN THE ZOOLOGICAL SOCIETY'S LIVING COLLECTION

THE KOALA (*Thascolarctos cinereus*). For many years it was deemed impossible to keep the Koala, or Native Sloth of Australia, alive in captivity. Great and persistent efforts, it was said, had been made by many persons in various part of the Australian Colonies to induce this curious little animal to submit to confinement. But as they never survived long, even under the most favourable conditions in Australia, it was hopeless to expect that we should ever see this animal living in London.

These prophecies, however, like other forebodings on more serious subjects, have turned out to be fallacious. In April, 1880, the Society acquired a living example of this animal in excellent health. It had been brought home from Australia along with a large barrel of the dried leaves of one of the gum-trees (*Eucalyptus*), upon which scanty diet, however, it appeared to have thriven well during the voyage. On being placed in a compartment of a room fitted up specially for it with branches to climb about upon, and supplied with fresh gum-tree leaves and a little bread and milk, it continued to prosper admirably, until it lost its life by an untoward accident.

The specimen had not been replaced until May last, when a second example, from which our Fig. (24) has been taken, was acquired.

The Koala is apparently confined to the south-east of Australia. It is reclusive in its habits, hiding in the day time in the dense foliage of the eucalypti or native gum trees, so that without the aid of the natives it is not

easily detected. By these, however, it is readily discovered, and captured by the aid of their waddies or throwing-sticks. It is exceedingly tenacious of life, clinging to the branches after being shot until perfectly dead.

The natives of Australia are said to be very fond of the flesh of the Koala, and readily join in the pursuit of it; they examine with wonderful rapidity and minuteness the

branches of the lofties, gum tree, and upon discovering a Koala, they climb the tree with as much ease and expedition as a European would mount a tolerably high ladder. Having reached the branches, which are sometimes forty or fifty feet from the ground, they follow the animal to the extremity of a bough, and either kill it with a tomahawk, or take it alive. From *Nature* **26**, 604, 19 October 1882.



FIG. 24.—The Koala.

REVIEW ARTICLE

Rheology of ices: a key to the tectonics of the ice moons of Jupiter and Saturn

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The polymorphic varieties of water ice are important rock-forming minerals in the outer Solar System. The Voyager 1 and 2 missions have shown that most of the ice moons of Jupiter and Saturn have had some form of tectonic or internal activity. Understanding of the tectonics and building reasonable models of the interior of these planets depend on our knowledge of the rheological properties and viscosity of high-pressure and low-temperature forms of ice.

WATER ICE, solid H_2O , is a unique rock-forming mineral in that it is the only one so close to a solid-liquid-vapour triple point at the temperatures and pressures of the Earth's surface. The crustal rock, ice, is of atmospheric origin and bears little relation to tectonics or to the dynamics of the Earth's interior: it is only when melting and retreating that ice caps and glaciers reveal sculptured landscapes and let the viscous asthenosphere isostatically adjust to the decreased load by postglacial rebound. Thus, whereas the global tectonics movement has led, in the 1970s, to an upsurge of interest in the rheology of rocks and minerals from tectonicians and solid-Earth geophysicists alike, the solid-state flow properties of ice are still investigated only by glaciologists and materials scientists. However, the recent Voyager 1 and 2 missions to the outer giant planets¹⁻⁴ may well have put an end to this state of affairs: while only 2 yr ago, the planetologists, solid-Earth geophysicists and tectonicians interested in comparative planetology studied only five rocky planets: Earth, Moon, Mercury, Venus and Mars, their range of interest has now expanded to include four major satellites of Jupiter and seven of Saturn, which the Voyager imagery transformed from mere specks into respectable planets (Table 1). Many of these 'new' planets exhibit superficial signs of past or present tectonic activity (such as faulting and rifting) or features that can be interpreted as surface expressions of internal activity (such as relaxation of impact craters and extrusion of younger terrain). The understanding and interpretation of these exciting features requires some knowledge of the rheological properties and viscosity of the rocks constituting the planets. Now, of these 11 new objects, ranging in size from that of an average asteroid to that of Mercury, only one (Io) is entirely composed of silicate rocks, one, although mostly rock, is heavily coated with ice (Europa), and all the others have a density and an albedo consistent with a content of 50 to almost 100 vol % of water. For the smaller bodies (the satellites of Saturn, except Titan and possibly Rhea) the interior temperatures and pressures are low enough for water to be frozen, and these planets consist mostly of the ice we are familiar with on Earth: ice I, which, however, is only one among 10 polymorphic varieties of crystalline ice⁵ (Fig. 1). The other higher pressure varieties might be found in the larger satellites depending on their thermal evolution, which in turn depends on the viscosity of the high-pressure forms of ice: a low interior viscosity will allow an efficient removal of heat by convection, whereas a high viscosity will prevent convection and possibly lead to partial melting and differentiation with formation of a silicate core.

Voyager has made us aware of the fact that the high-pressure and low-temperature polymorphic varieties of ice are essential rock-forming minerals in the outer Solar System, as a consequence their metamorphism and solid-state flow properties are fast becoming an object of concern for planetologists inter-

ested in the tectonics and the interior dynamics of solid planets. The time may have come for a review of the tectonic and dynamic problems of the icy satellites, relative to the rheology of ices, from fracture to high-temperature creep, as well as for an assessment of what we know and what we should know about the rheological properties of water ice in conditions of low temperatures and high pressures.

Surface tectonics and mechanical properties

Tectonic features seen on the surfaces of Jupiter's and Saturn's ice moons can be divided into three groups: large-scale lineaments thought to be fractures; impact craters, sometimes surrounded by multi-ringed structures and whose topography can be relaxed if the internal viscosity is low enough, and finally expanses of young smooth terrain, with few craters, resurfacing large areas and possibly resulting from the extrusion of internal material.

Europa displays a highly reflecting surface criss-crossed by a network of low albedo lineaments, with very little relief, extending over long distances^{1,2}. Analysis of the pattern⁶ reveals that the dark lines appear to be radial and concentric around a point situated at the antipodes of the sub-Jupiter point, while many of the lines intersect with an angle of 60–70°. These observations are consistent with an interpretation of the lineaments as conjugate shear fractures in Europa's crust; the pattern of fractures may have formed as a result of planet-wide tidal distortion of the ice crust during despinning and synchronization of the satellite. It has also been suggested that the fractures may have been caused by global expansion due to dehydration of hydrated silicates thought to constitute the mantle⁷. Long,

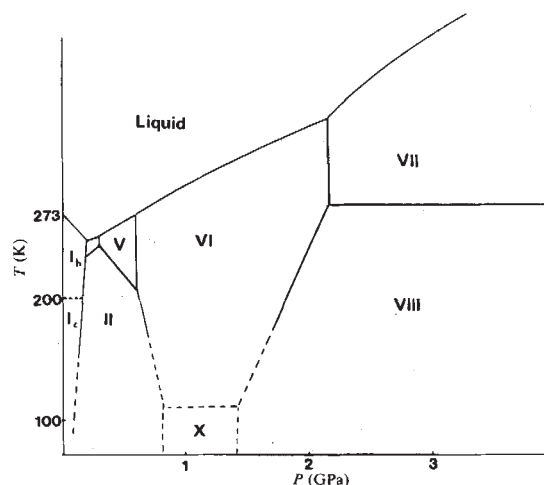


Fig. 1 Phase diagram of water ice (from ref. 5).

Table 1 Parameters for the satellites of Jupiter and Saturn

Planet	Radius <i>R</i> (km)	Average density ρ	Gravity at surface g (cm s ⁻²)	Albedo	Calculated volume fraction of ices, <i>f</i>	Varieties of ices possibly present	Calculated pressure at centre, <i>P</i> (MPa)
J1 Io	1,819	3.53	179	0.6	0		
J2 Europa	1,563	3.03	132	0.6	0.2	I	
J3 Ganymede	2,638	1.93	142	0.4	0.7	I, II, V, VI, VII	
J4 Callisto	2,424	1.79	122	0.2	0.7	I, II, V, VI, VII	
S1 Mimas	196	1.19	6.6	0.6	0.9	I	8
S2 Enceladus	250	1.2	7.7	0.9	0.9	I	11
S3 Tethys	530	1.21	14.7	0.8	0.9	I	39
S4 Dione	560	1.43	22.4	0.62	0.8	I	90
S5 Rhea	765	1.33	28.4	0.65	0.85	I, II	145
S6 Titan	2,575	1.88	135	0.2	0.7	I, II, V, VI, VII	
S8 Iapetus	730	1.16	14.9	0.5/0.05	0.9	I	100

The radius *R*, density ρ and albedo values are taken from refs 1 and 4. The volume fraction of ices is calculated: $f = (\rho_s - \rho) / (\rho_s - \rho_w)$ with $\rho_s = 3.5$ density of silicates (non-hydrated) and ρ_w average density of water (ices) taken to be 0.93 for small bodies and 1.2 for large bodies²⁵. The pressure at the centre is calculated for small bodies assuming that the density is the same at all depths and equal to the average density¹⁶. No values are given for the pressures at the centre of large bodies, as they depend on the thermal evolution model and the resultant structure of the interior. Ice I would be stable down to a depth of 22 km on Earth and 150 km on Ganymede.

straight lineaments on Enceladus also appear to be fractures or grabens. Clearly some knowledge of the fracture strength of ice at low temperatures would be helpful in building more quantitative models and assessing their plausibility.

Impact craters are possibly the most widespread tectonic surface features throughout the Solar System and measurement of their density is used as a means to estimate the relative ages of various parts of planetary surfaces. Moreover, study of the ejecta may provide information about the subsurface layers: small craters on Ganymede often eject dark rays as they tap the silicate-rich regolith only, whereas larger craters penetrating deeper levels of purer ice are surrounded by white rays⁸. High-pressure polymorphs of ice may be formed during the impact, they can be indefinitely preserved at temperatures lower than 120K but will transform to cubic ice *I_c* if warmed to higher temperatures: it could, in principle, be possible to estimate thermal gradients near the surface by using remote sensing IR spectroscopy to measure the retained fraction of high-pressure ices as a function of the crater size⁹ (larger craters tapping deeper, warmer layers of ice should produce ejecta transforming more easily into ice *I_c*).

The size distribution and topography of craters depend directly on the viscosity of subsurface and deep ice and its variation with depth. Craters created by impact on viscous icy bodies will relax and eventually lose their topographic relief; the relationship between viscous relaxation of topography and the variation of rheological properties with depth in an ice crust has been studied by Parmentier and Head¹⁰. They discovered that the underabundance of large craters relative to small craters systematically found in icy bodies as compared with silicate bodies can be accounted for by a model where viscosity decreases with increasing depth. Solid-state viscosity being thermally activated, this implies that the inside of the planet is (or was) warmer with increasing depth. The relaxation time for the crater cavity is proportional to the effective viscosity and inversely proportional to the diameter *D*, but as a large crater interest a deeper (less viscous) zone, the effective viscosity also decreases with increasing *D*, so that the relaxation time decreases faster than $1/D$. Using an exponential variation of viscosity with depth, it is found¹⁰ that the cavity of a 100-km diameter crater should relax about 1,000 times faster than the cavity of a 10-km diameter crater. On a small, cold body such as Mimas a large crater has retained its sharp and crisp shape whereas a larger crater on Tethys has flattened out leaving only a circular scar, suggesting that the inside of Tethys has been warmer, hence less viscous. Extreme cases are probably represented by Valhalla crater on Callisto and the ghost craters ('palimpsests') on Ganymede. Floors of craters wider than 10 km are often convex upwards (which is unusual for craters on silicate surfaces); the existence of domed crater floors has

been found to be consistent with relaxation on a deep layer whose viscosity is uniform or decreases with increasing depth¹⁰. Large craters are often surrounded by multi-ringed structures spreading far from the centre: a typical example is the relaxed crater Valhalla on Callisto. These spectacular structures have been explained^{11,12} as concentric grabens or rifts in the brittle lithosphere formed in response to a state of radial tension created at the base of the lithosphere; the tension is due to the collapse back-flow of a viscous asthenosphere under the large deviatoric stresses generated by the crater formation. Maxwellian relaxation times for ice creeping at high temperatures relative to the melting point can be short enough to allow significant solid-state flow to occur.

The size of the crater cavity (before collapse) depends also on the mechanical properties of ice but in different conditions: rather than high-temperature viscosity, the relevant parameter is here the dynamic tensile strength of the crust as the cavity is mostly formed by spalling. Recent laboratory experiments¹³ have shown that the admixture of silicates (fine sand) to ice considerably strengthens the ice and results in smaller craters. Lange and Ahrens¹⁴ have estimated the dynamic tensile strength of ice and ice-silicate mixtures at temperatures between -43 and -33 °C: tensile stresses were induced in the sample by shock of an impactor plate driven by a light gas gun. The strain rates achieved were $\sim 10^3$ s⁻¹. The tensile strength (peak tensile stress at which samples spall or fragment) is

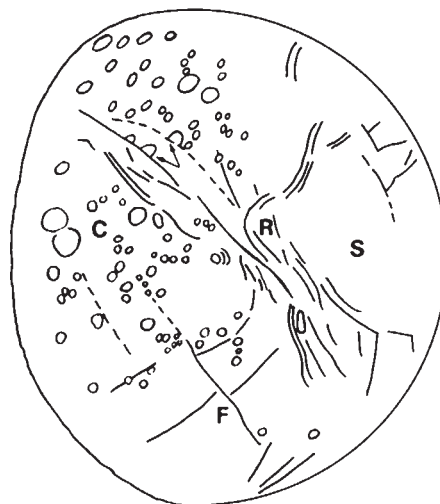


Fig. 2 Schematic structural map of Enceladus. C, cratered terrain; F, fractures or grabens; S, smooth terrain; R, ridges; craters cut by smooth terrain are shown by arrows.

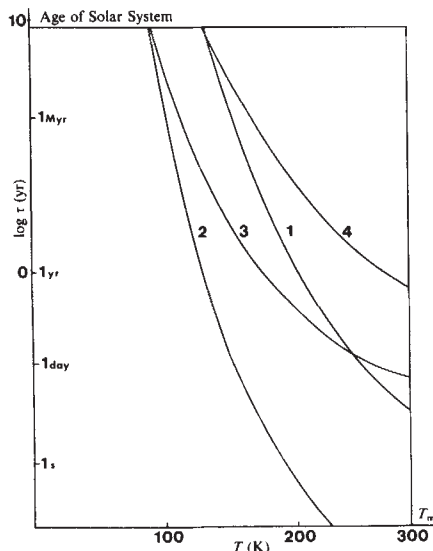


Fig. 3 Variation of Maxwellian relaxation time of ice I, $\tau = \sigma / \dot{\gamma} \mu$ as a function of temperature, for various creep mechanisms. 1, Proton rearrangement at high stresses ($\sigma = 400 \text{ bar} \approx 10^{-2} \mu$); 2, proton rearrangement at low stresses ($\sigma = 4 \text{ bar} \approx 10^{-4} \mu$); 3, diffusion creep for a grain size of 0.01 cm; 4, diffusion creep for a grain size of 1 cm. The creep laws $\dot{\gamma} = f(\sigma, T)$ are taken from ref. 38 (equation (4a) for 1, (4b) for 2, (5) for 3 and 4).

16–17 MPa for pure ice, 18–20 MPa for ice–5 wt% sand and 22–25 MPa for ice–30 wt% sand. As the upper layers of Ganymede and Callisto can be expected to contain silicate particles, it is important to know the mechanical properties of ice–silicate composites.

Crater densities and size distribution are unusually far from being uniform on the surfaces of ice moons; lack of uniformity in the crater density over the surface of one satellite implies different ages for the terrains: less cratered areas are younger than heavily cratered ones and presumably result from some internal activity. Analysis of Voyager 1 imagery of the saturnian satellites Rhea, Dione, Mimas and Tethys^{15,16} suggests that they have undergone various degrees of crustal resurfacing.

Enceladus surface shows a diversity of terrains and tectonic features⁴ surprising in such a small body (Fig. 2): more or less heavily cratered terrains of various ages, smooth plains devoid of craters but cut by long lineaments (probably fractures), ridges and valleys. The young, smooth terrain covers parts of the old cratered terrain (some craters are half-covered at the boundary between the two). There seems to be little doubt as to the extrusive nature of the smooth terrain although the nature of the source of energy driving the ice volcanism is far from being clear. Radioactive decay or accretion do not provide enough heat to melt the ice¹⁶ and tidal dissipation heating may not be sufficient either¹⁷. To overcome these difficulties, it has been proposed that volcanism is due to melting and upwards migration of lower melting point ammonia clathrate hydrates^{16,18,19}. Klinger²⁰ proposes another heating mechanism: if originally, ice has accreted as amorphous ice, the heat release during the irreversible transition to cubic ice I_c around 153 K might be enough, together with a modest tidal heating, to reach the melting point. Finally, note that all thermal models depend on the uncertain values adopted for the viscosity of ice I (and possibly II) at low temperatures.

The largest of the jovian and saturnian ice moons is Ganymede—slightly larger than Mercury—which is also the one which exhibits the most unusual and fascinating tectonics. Areas of primitive, cratered, dark terrain are separated by wide swaths of bright grooved terrain or cut up in a puzzle of polygons by narrow channels or wedges of the same bright terrain. The overall pattern is vaguely reminiscent of a map of the Earth with continents, rifts and faults, but the topographic relief is generally weak. The grooves in the bright terrain belong to

various types^{21,22}, many are subparallel, broad U-shaped troughs, a few hundred metres deep²³. Interpretations of the tectonics of Ganymede have recently been reviewed by Parmentier *et al.*²⁴, the present view is that there is little evidence for terrestrial-style plate tectonics with rifting and lateral motions and that the tectonics can be best explained by models of global expansion of the surface (5–7%)²⁵ due to internal phase changes of the ice to less dense polymorphs (or even melting during differentiation); this would cause rifting and extrusion of the underlying cleaner material, flooding dark terrain. It is also possible that broken-up polygons of dark terrain could founder into purer less dense ice, thus increasing the bright areas. The style of tectonics at the surface depends on the internal structure and dynamics, which in turn depends on the viscosity.

Internal dynamics and viscosity

The problem of building a thermal model and predicting the internal dynamics (has solid-state convection been active in the past or is it active now?) considerably depends on the size of the body. In all cases, it is generally assumed that the accretion was homogeneous and that the primitive material is a mixture of ice and silicates in various proportions. The sources of heat considered are usually radioactive decay and accretion. In small bodies (all saturnian moons except Titan and possibly Rhea), the only stable variety of ice is ice I²⁶. The Rayleigh criterion for the onset of convection depends on the viscosity of ice I. Ellsworth and Schubert¹⁶ assume that, at temperature T (K), the kinematic viscosity of ice I in $\text{m}^2 \text{s}^{-1}$ is given by:

$$\nu = 0.139 \exp\left(\frac{7,214}{T}\right)$$

They found that ice can never melt in these small bodies and that convection by subsolidus creep is inevitable at some stage if the planet is not too small: Dione and Rhea are still probably active and convecting now, Tethys should have been very active in the past but is probably in the conductive stage now, whereas the small Mimas is cold and isothermal.

The large icy bodies (Ganymede, Callisto and presumably Titan) present a more complex problem because high-pressure ices can exist deep inside. Two scenarios can be built: if the viscosity of high-pressure ices (VIII, VII, VI, V, III, II according to the depth and the temperature reached) is high enough to prevent subsolidus creep and convection, melting and differentiation of the planet can occur²⁷, silicates sink to form a rocky core thus taking the place of the highest pressure ices. Eventually the planet would freeze again due to convection in the ice I lithosphere²⁸ and diapiric descent of ice in the liquid mantle by lithospheric instability^{29,30} to end up as a differentiated body with a silicate core and a mantle consisting of layers of various pure ice polymorphs. However, for ice viscosities lower than 10^{16} – 10^{18} P, melting would probably not occur and for near-melting temperature viscosities close to 10^{14} P a solid-state body would be primarily solid³⁰ and convecting; this is probably the case for Ganymede and Callisto. Schubert *et al.*³¹ have reached the same conclusion by taking for high-pressure ices a creep law similar to an empirical law for ice I, giving a viscosity of 10^{14} P near melting point for a shear stress of 0.1 MPa. Experimental measurement of the viscosity of ice VI near melting point by Poirier *et al.*³² confirms the validity of this hypothesis. Ganymede could be largely undifferentiated, with only its outer layers molten by accretional heat (and refrozen) and a small core formed by the silicate particles of the outer layer which have sunken through the solid mantle³¹. Callisto, on the other hand, may consist entirely of a homogeneous ice–silicate mixture³¹.

Rheology of ice I

Ice I is the essential constituent of the smaller ice moons and of the outer layers of the large ones; it is also the only polymorph of ice found on Earth, at least in its hexagonal variety I_h, with a wurtzite-type lattice of oxygen ions (puckered hexagonal rings of H₂O molecules stacked in a ABAB sequence)³³.

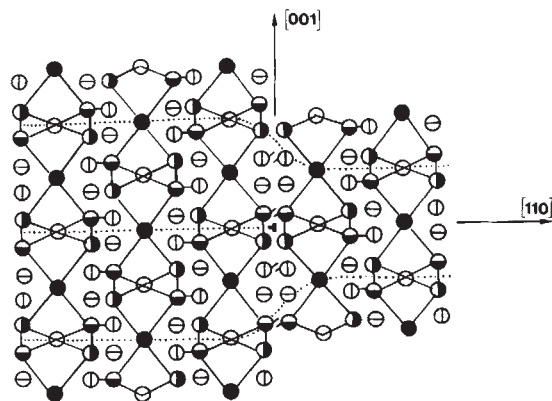


Fig. 4 Edge dislocation with Burgers vector [001] on glide plane (110) in ice VI structure. Motion of the dislocation line ($\perp$) (perpendicular to sheet) in the glide plane creates plastic deformation by slip⁵⁸. Symbols refer to oxygen ions in six parallel (110) planes: from front to back, $\odot$, $\bullet$, $\ominus$, $\oplus$, $\circ$, $\ominus$. The oxygen atoms shown by the closed and empty circles are situated at the centre of the tetrahedra forming the [001] chains.

The rheological properties of ice I_h have been well reviewed elsewhere³⁴⁻³⁹ and only the most important results are summarized here. Ice is one of the strongest materials known³⁸ if the comparison is made in terms of stresses normalized to the shear modulus and temperatures normalized to the melting temperature: at $T = 0.9 T_m$ and $\sigma = 10^{-4} \mu$ a typical creep rate is $\dot{\gamma} = 10^{-10} \text{ s}^{-1}$, compared with $\dot{\gamma} = 10^{-7} \text{ s}^{-1}$ for covalent elements (Si, Ge) and $\dot{\gamma} = 3 \times 10^{-4} \text{ s}^{-1}$ for alkali halides or fcc metals in the same conditions³⁸. Preliminary work by W. B. Durham, H. C. Heard and S. H. Kirby (personal communication) on the mechanical behaviour of ice at low temperatures (77–195 K) and confining pressures up to 100 MPa, shows that at 195 K ($0.7 T_m$) ice behaves in a totally brittle fashion, for a strain rate $\dot{\epsilon} \approx 4 \times 10^{-4} \text{ s}^{-1}$, even under 100 MPa confining pressure; it becomes plastic only for a strain rate of $4 \times 10^{-6} \text{ s}^{-1}$. For the same strain rate and confining pressure, but at 113 K, the ice is still brittle. When subjected to high dynamic stresses, ice fractures by cleavage even at its melting point and no ductile fracture ever occurs⁴⁰. The known fracture properties of ice have been recently reviewed and the mechanisms displayed in fracture-mechanism maps^{40,41}; we will consider here only the creep properties. Solids creep by stress-directed diffusional flow or by motion of dislocations⁴². Diffusional flow is controlled by the diffusion of vacancies in bulk (Nabarro–Herring creep) or in grain boundaries (Coble creep), it prevails only at high temperatures, low stresses and small grain sizes⁴³ that are seldom obtained in natural pure ice.

Dislocation creep mechanisms, not surprisingly, depend on the peculiar crystallographic structure of ice: the protons are disordered and there must be two protons per oxygen atom and one proton per hydrogen bond between neighbouring oxygens (Bernal–Fowler rules). As a result, a dislocation moving through the ice lattice with immobile disordered protons cannot move without creating defects (Bjerrum defects) violating the Bernal–Fowler rules, consisting in bonds with two protons or none⁴⁴. The energy of creation of the defects being quite high (0.64 eV per bond), it follows that it is extremely difficult to move a dislocation when the protons are immobile, that is at very low temperatures: at 0 K the stress to move the dislocation would be of the order of the theoretical shear strength: 0.1μ (ref. 38). At higher temperatures and low stresses ($\sigma < 2 \times 10^{-4} \mu$) the dislocation moves by nucleating a kink pair where the local proton configuration in its path is favourable—where a Bjerrum defect has diffused. Consideration of hollow or non-crystalline dislocation cores⁴⁵ would make the proton rearrangement rules less stringent. A creep law can be calculated³⁸ with a power law dependence on stress (Glen's law) and an activation energy equal to the energy for proton rearrangement.

Adjustment of experimental creep data gives the empirical law⁴⁶:

$$\dot{\gamma} = 3.9 \times 10^{20} (\sigma/\mu)^3 \exp - (80 \text{ kJ}/RT) \text{ s}^{-1} \text{ for } T < -8^\circ \text{C}$$

$$\dot{\gamma} = 3 \times 10^{28} (\sigma/\mu)^3 \exp - (120 \text{ kJ}/RT) \text{ s}^{-1} \text{ for } T > -8^\circ \text{C}$$

At higher stresses ($\sigma > 2 \times 10^{-4} \mu$) or temperatures below $0.5 T_m$ the power law breaks down and the rate limiting process becomes kink nucleation (and eventually defect creation). Deformation mechanism maps³⁸ display the temperature and stress domains where the various mechanisms are active. Figure 3 shows the variation of the Maxwell relaxation time $\tau = \eta/\mu = \sigma/\dot{\gamma}\mu$ with temperature for the high-stress and low-stress proton rearrangement mechanism compared with the diffusional flow mechanism.

The effect of impurities on the creep of ice may be interpreted in terms of Bjerrum defects and proton rearrangement. HF dissolved in ice greatly enhances its creep rate; this was attributed to the increased concentration of L-Bjerrum defects (no proton on the bond) allowing the dislocation to move faster⁴⁷, and to the operation of an easier multiplication mechanism for dislocation⁴⁸. NH_3 in solution apparently has the inverse effect and hardens the ice⁴⁹ but no convincing reason has yet been found for this effect, which could be highly relevant to the saturnian ice moons. In general the influence of NH_3 or CH_4 on the creep of ice should certainly be studied more thoroughly.

Another subject which has been investigated is the influence of a dispersion of sand on the creep of ice, because all ice moons in their primitive undifferentiated state are probably mixtures of ice and silicates in various proportions. Apart from inhibiting dynamic recrystallization and grain growth⁴⁹ which are effective softening processes in pure ice⁵⁰, mixing of sand with ice causes dispersion hardening⁵¹: the creep rate decreases and the creep activation energy increases⁴⁹ with increasing volume fraction of sand.

Cubic ice I_c has a sphalerite (zincblende) lattice of oxygen ions (puckered hexagonal rings of H_2O molecules stacked in an ABC ABC sequence)³³, it is stable only at low temperatures and transforms to ice I_h in the vicinity of 200 K, whereas ice I_h persists in a metastable state when cooled down into ice I_c field³³. All high-pressure polymorphs of ice first transforms to ice I_c when they are brought to atmospheric pressure at low temperatures; amorphous ice, obtained by condensation of water vapour at low temperatures also first transforms to ice I_c when heated. Cubic ice might therefore be present in ice moons at some stage of their evolution and it would be useful to know something about its mechanical properties. Unfortunately, experimental investigations have so far been limited exclusively to hexagonal ice and even then, to the high-

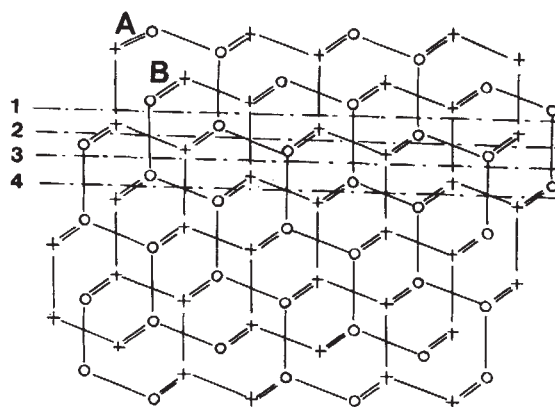


Fig. 5 Structure of Ice VII. The two interpenetrating diamond cubic lattices are shown in projection on a (110) plane. The hexagonal puckered rings of oxygen ions in {111} planes are shown edgewise. Four possible {111} glide planes are shown: 1 and 3, shuffle set of lattice A and lattice B; 2, shuffle set of lattice A, glide set of lattice B; 4, glide set of lattice A, shuffle set of lattice B.

temperature domain close to the melting point ($T > -50^\circ\text{C}$ or $T > 0.8 T_M$) relevant to conditions found on Earth. Mechanical properties of ice I_c can therefore be only the subject of educated guesses, based on what we know of hexagonal ice.

The solids whose mechanical properties normalized to elastic moduli and melting point are most similar to those of ice I_h are the strong covalent semiconductors Si and Ge (refs 38, 46). The atoms of these crystals are tetrahedrally coordinated in a diamond cubic lattice as the oxygen atoms of ice I_c (with covalent bonds instead of hydrogen bonds). There are therefore good reasons to think that, concerning its mechanical properties, ice I_c could be even more similar to silicon and germanium than ice I_h , in other words, ice I_c should be a very strong solid at the temperatures where it is stable.

Rheology of high-pressure ices

Very little is known of the physical properties of the high-pressure polymorphs of ice and most of the work so far has been largely concerned with refinements of the crystallographic structures. What follows will therefore be mostly speculations on the plastic properties of the varieties of ice which are most likely to be met in the larger ice moons (ices II, VI, VII and VIII) whose stability fields occupy most of the P, T plane (Fig. 1).

The structure of ice II is rather similar to that of ice I (ref. 52), with puckered hexagonal rings of H_2O molecules stacked in columns, but the columns are packed in a more economical manner than in ice I thus achieving greater density ($\rho = 1.17$ at room pressure and $T = 100\text{K}$). Ice II is rhomboedral and one could expect that glide of the dislocations on the close-packed planes would occur in much the same way as glide on the basal plane of hexagonal ice I_h , except for one very important difference: the protons in ice II are ordered^{52,53} whereas they are disordered in ice I. The motion of dislocations in the lattice of oxygen ions and ordered protons would certainly cut bonds but it would not have to create energetic defects as in ice I, because Bjerrum defects can be defined only if the protons are disordered and obey Bernal-Fowler rules. In most cases, creep of ice I is controlled by proton rearrangement mechanisms in front of the advancing dislocation; this step would be unnecessary in ice II and the viscosity of ice II might be lower than that of ice I in equivalent conditions. Preliminary results on the effective viscosity of ice II and III have recently been obtained by K. Echelmeyer and B. Kamb (personal communication) by extrusion of the ice through an orifice in the wall of the pressure vessel. They found that ice II is more viscous than ice I_h at the same temperatures ($T > 193\text{K}$); it is, however, difficult to compare the viscosities of ice I_h and II at equivalent temperatures (same value of T/T_m) as ice II has no melting point (it transforms to ice III or V, see Fig. 1). At the same temperature, ice III is found to be three orders of magnitude softer than ice I_h .

Ice VI has a very large stability field and can be formed directly from liquid water at room temperature. Its structure consists of two identical and independent frameworks of chains of tetrahedrally linked water molecules mutually interpenetrating, constituting a 'self-clathrate'⁵⁴. The lattice is tetragonal with the chains in the c direction. Within one framework, the H bonds are longer between chains than between nearest neighbours along a chain. Simple inspection of the structure suggests that glide could easily occur on $\{110\}$ planes, in the $[001]$ direction, with dislocations cutting only the weaker bonds between chains (Fig. 4). The protons, disordered in ice VI, order at temperatures lower than -160°C to give ice VI' (or

X)^{52,55}. The viscosity of ice VI has recently been directly measured by Poirier *et al.*³². Ice VI was formed at room temperatures by compressing water in a copper gasket between sapphire anvils. The creep rate of ice flowing radially outwards down the hydrostatic pressure gradient was measured by following optically the motion of deformation markers; pressure was calibrated by using alkali halides phase transformations. At temperatures close to the melting point and shear stresses of several hundred bar, the viscosity was found to be of the order of 10^{13} P. As the elastic moduli of ice VI are still unknown, it is not yet possible to compare the viscosities of ice VI and ice I for the same values of σ/μ . We can only note that a viscosity of $\sim 10^{13}$ P close to the melting point of ice I would be found for stresses of only a few tens of bar, but the shear modulus of ice I is unusually small, and it is not unreasonable to expect the more close packed varieties to have higher moduli. Ice VII is the densest of all known polymorphs of ice ($\rho = 1.5$ at atmospheric pressure and $T = 100\text{K}$). Its crystallographic cell is cubic and the lattice can be described as consisting in two independent interwoven lattices of cubic ice I_c (ref. 56) (Fig. 5) with disordered protons. At low temperatures the protons order to give ice VIII (ref. 57) where the water molecule dipoles are oriented in opposite senses on each lattice (antiferroelectric order). Proton rearrangement mechanisms are therefore expected to play a part in the deformation of ice VII as they do in ice I. As mentioned above, ice I_c has a diamond cubic lattice of oxygen ions and can be compared with the covalent crystals silicon and germanium. Glide in this structure can be envisioned on two sets of $\{111\}$ dense planes⁵⁸: dislocation gliding on the 'glide set' (cutting the hexagonal puckered rings) cuts more bonds but can dissociate with a low energy stacking fault, whereas dislocations moving on the 'shuffle set' (between the planes of the rings) would cut less bonds but a reorientation of the bonds would be necessary. In ice VII, the $\{111\}$ slip planes could belong to the glide set of one of the ice I_c lattices and the shuffle set of the other, or to the shuffle set of both but it is impossible to have slip on both glide sets (Fig. 5). As the glide set is generally the easier one in diamond cubic structures, it is reasonable to expect that ice VII should be more difficult to deform than ice I_c as there would be more bonds to cut per unit area of slip plane and at least one shuffle set would necessarily be involved.

The review of the problems of tectonics and internal dynamics of the ice planets of the outer Solar System has, it is hoped, demonstrated that most of the answers depend on a better knowledge of the rheological properties of terrestrial ice at low temperatures and of high-pressure ices at all temperatures. The elastic moduli of high-pressure ices should be measured, perhaps by Brillouin scattering techniques in diamond anvil cells. Viscosity of ice I at temperatures lower than -50°C could be investigated by traditional techniques and deformation maps completed. The field of rheological properties of high-pressure ices is still fallow and wide open. The physics of dislocations in hydrogen-bonded systems with fascinating structures poses many interesting problems; the role of the order-disorder transition in protons is only one of them. A whole field of research for materials science, which hitherto could have been deemed of academic interest, can now yield urgently needed data.

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ARTICLES

Deuterium excess in an East Antarctic ice core suggests higher relative humidity at the oceanic surface during the last glacial maximum

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A continuous deuterium excess profile ($d = \delta D - 8\delta^{18}O$) was obtained from the Dome C ice core spanning ~32,000 yr. It exhibits significant variations over this period. In particular, d was 4.5% lower around 18,000 yr BP than over the past 7,500 yr, interpreted as reflecting higher relative humidity (estimated ~90%) over the oceanic areas providing moisture for Antarctic precipitation.

As a result of fractionation processes occurring in the atmospheric water cycle, there is a generally well obeyed linear relationship between δD and $\delta^{18}O$ in precipitation^{1–3} (δD and $\delta^{18}O$ are expressed in ‰ versus VSMOW). Craig¹ determined the meteoric water line, $\delta D = 8\delta^{18}O + 10$, as the locus of the isotope composition of worldwide freshwater sources. Dansgaard² defined the deuterium excess parameter, $d = \delta D - 8\delta^{18}O$ and pointed out that d can apparently be used to identify non-equilibrium processes.

The $\delta D - \delta^{18}O$ relationship in ancient well-dated precipitation has only been determined in a few studies. From selected samples of the Camp century ice core (Greenland)⁴, covering nearly the past 100,000 yr, a close linear correlation $\delta D = 8\delta^{18}O + 12$ independent of the time of formation was found. Glacial and interglacial samples from the Byrd core (Antarctica)⁵ spanning more than 75,000 yr fit a curve with a slightly different slope, that is $\delta D = 7.9\delta^{18}O$. In these two cases, no climatological information over and above that deduced from the $\delta^{18}O$ profile was obtained from the $\delta D - \delta^{18}O$ relationships. Pleistocene Saharian⁶ groundwaters show d values of 5‰ (ref. 6) which were interpreted in terms of relative humidity changes^{6,7}. Finally, isotopic data on fluid inclusions and host speleotherms provide an indirect indication of a relative deuterium deficiency during Pleistocene glacial periods⁸.

We now present and discuss a continuous d record obtained from the Dome C ice core recently drilled in East Antarctica⁹ and covering the late Pleistocene and Holocene periods.

Isotopic results

A thermal core drilling to 906 m depth was carried out at Dome C (74°39' S; 124°10' E) during the 1977–78 Antarctic field

season⁹. The smoothed $\delta^{18}O$ continuous profile is shown in Fig. 1a. The core was dated using a constant accumulation rate over the Holocene (0.037 m ice yr⁻¹ down to 381 m) and then a lower value as suggested by comparison with typical well-dated climatic events existing in deep-sea core isotopic profiles. According to this model, the age of the bottom of the core is ~32,000 yr. Four main isotopic stages can be distinguished in the $\delta^{18}O$ profile (Fig. 1): the Holocene stage (stage 1), the isotopic transition (stage 2), the stage with minimum $\delta^{18}O$ values (stage 3) and the earlier stage (stage 4). Note that these stages, defined here for practical purpose, are not similar to that used in the description of the $\delta^{18}O$ records obtained from deep-sea cores¹⁰. Despite some imprecision in dating, stage 3 certainly includes the last glacial maximum centred around 18,000 yr BP¹¹.

Deuterium determinations were simultaneously performed¹² on the same samples used for $\delta^{18}O$ (ref. 9), with an accuracy (1 σ) of 0.5‰ and 0.15‰ on δD and $\delta^{18}O$ respectively, leading to an accuracy of 1.3‰ for individual d determinations. A procedure similar to the one applied for ^{18}O values⁹ was then followed to obtain the smooth d profile presented in Fig. 1b. This curve shows relatively uniform conditions over the past 7,500 yr ($\bar{d} = 8.3$ ‰) with a period showing significantly lower values (down to 7.3‰) centred around 4,000 yr BP. Note the d decrease in the early Holocene (7,500–10,000 yr BP) with no simultaneous $\delta^{18}O$ change, the two curves presenting an apparent time-lag of ~2,500 yr. A rapid d decrease is then observed during the isotopic transition (stage 2). Minimum d values of ~4‰ are observed in the coldest part of the last ice age around 18,000 yr BP (later part of stage 3). From this point down to the early part of stage 3, a d increase is observed

without any corresponding change in $\delta^{18}\text{O}$. Significantly higher values (up to 7‰) are displayed in the later part of stage 4 with a further decrease down to the bottom of the core.

This continuous d profile was completed in two ways: (1) as a basis for modern conditions, detailed δD and $\delta^{18}\text{O}$ measurements (~ 10 samples yr^{-1}) were carried out on samples taken from two different pits representing snow accumulated over the past two decades. The mean d values are $9.8(\pm 0.2)\text{‰}$ and $9.6(\pm 0.2)\text{‰}$ respectively; (2) additional results were obtained from detailed analyses of 19 ice core pieces (with typically 40 successive samples for 60-cm long increments). Figure 2 shows the results for two of them, one for the Holocene period (315 m) and the other during the glacial maximum (541 m). The deuterium profile inside core increments, provides information about the homogenization of isotopic signals in firn and ice by water vapour diffusion¹³ which will be discussed elsewhere. In a given piece, the d variability is of the same order of magnitude as the accuracy of individual d determinations, giving a very high accuracy (0.2–0.3‰) for the mean d values calculated for each increment. These mean values given in Fig. 1b (stars) confirm the d changes deduced from the smoothed continuous profile and there show that the accuracy along the d profile is around 0.2–0.3‰.

Interpreting the deuterium excess profile

We will focus the present discussion on the major d change of $\sim 4.5\text{‰}$ observed between the past 7,500 yr and the full glacial period. To this end, this term, Δd_i , is written as follows:

$$\Delta d_i = \Delta d_L + \Delta(dp_i - d_L) + \Delta(d_s - dp_i) + \Delta(d_i - d_s) \quad (1)$$

where d_L , dp_i , d_s and d_i are the d values (‰) of the sea-surface water, the first precipitation in the given air mass, the snow at time of precipitation and the ice core samples respectively. The Δ s refer to the differences between the two considered periods. The first precipitation is taken as an intermediate step of the discussion for practical purposes: on the one hand, $dp_i - d_L$ depends only on the source characteristics as discussed below; on the other hand, $d_s - dp_i$ is directly related to the $\delta\text{D} - \delta^{18}\text{O}$ slope, s , between the first precipitation and the snow precipitation points (close to the meteoric water line) through $d_s - dp_i = (s - 8)(\delta^{18}\text{O}_s - \delta^{18}\text{O}_{pi})$ where $\delta^{18}\text{O}_{pi}$ and $\delta^{18}\text{O}_s$ are the oxygen-18 content of the first precipitation and of the snow respectively.

The deuterium excess change of oceanic water, Δd_L , was calculated similarly to the method followed by Dansgaard and Tauber¹⁴ for $\delta^{18}\text{O}$ assuming that precipitation 18,000 yr ago had a d value of 4 as is suggested by the Dome C record. This procedure gives a Δd_L of 0.1 which can be neglected in Δd_i .

It has been suggested¹⁵ that condensation and evaporation processes possibly affect the d value of the snow in the surface layers. A preliminary study carried out at South Pole¹⁶ effectively shows a slight d decrease of the firn samples (9.1 ± 0.2) compared with the precipitation samples (11.1 ± 0.4). At Dome C, a d decrease of the same order is observed between the surface samples (9.7 ± 0.2) and the mean value over the

past 7,500 yr (8.3). In the present discussion, data relative to this entire period will be compared with that relative to glacial conditions so that a possible influence of these processes which would only affect the top of the snow column can be ruled out ($\Delta(d_i - d_s) = 0$).

The isotopic content of the first precipitation δP_i can be derived from the model⁷:

$$\delta P_i = \frac{\alpha_e(1 + \delta_L)(1 - k)}{\alpha_e(1 - kh)} - 1 \quad (2)$$

where δ_L (δD or $\delta^{18}\text{O}$) is the isotopic content of the oceanic source, α_e and α_c are the isotopic fractionation coefficients at the evaporation and first condensation temperature T_e and T_c respectively, h is the relative humidity close to the oceanic surface, defined as the ratio of the specific humidity at height z (generally $z = 10$ m) to the saturated specific humidity at the sea-surface temperature. k depends on the ratio of the molecular diffusivities in air of the isotopic molecule (HDO or H_2^{18}O) and of H_2^{16}O , and on the windspeed with $k_D = 0.88k^{18}\text{O}$ whatever the evaporation regime. As δ_L , $\beta = \alpha_c/\alpha_e - 1$ and k are always very small (< 0.02), it becomes:

$$dp_i - d_L = (\beta_D - 8\beta_{18\text{O}}) + 7.12k_{18\text{O}}(1 - h) \quad (3)$$

$\beta_D - 8\beta_{18\text{O}}$ depends on both T_e and T_c and is, assuming a standard atmosphere ($dT/dz = -6.5^\circ\text{C km}^{-1}$), very closely approximated by $(13.6(1 - h) - 0.4) \times 10^{-3}$ over large ranges of T_e (0 – 30°C) and $T_c - T_e$ (0 – 12°C). In the Brutsaert's model of evaporation which quantitatively describes the isotope distribution in the atmosphere above a water surface¹⁷, $k^{18}\text{O}$ is practically uniform (~ 6) in the smooth regime of evaporation which prevails when the windspeed U (taken also at 10 m above the sea-surface) is $\leq 7 \text{ m s}^{-1}$. The appearance of the rough regime ($U > 7 \text{ m s}^{-1}$) is accompanied by a discontinuity with $k^{18}\text{O} = 2.8$ at 7 m s^{-1} (‰) followed by a regular increase very well fitted by $k^{18}\text{O} = 0.285U + 0.82$.

For present day sea-surface waters, d_L is close to zero so that dp_i can be expressed as a function of h and $k^{18}\text{O}$ alone. As 95% of the modern ocean surface is smooth¹⁸, a regime for which $k^{18}\text{O}$ remains constant, dp_i is mainly determined by the relative humidity close to the oceanic surface. With $d = 10$, a relative humidity of 81% is inferred which is very close to the value of 80% that actually prevails over the ocean surface¹⁹ giving a high degree of confidence in the above isotopic model.

From the above considerations, the d change observed in ice Δd_i is:

$$\Delta d_i = \Delta((13.6 + 7.1k^{18}\text{O})(1 - h)) + \Delta((s - 8)(\delta^{18}\text{O}_s - \delta^{18}\text{O}_{pi})) \quad (4)$$

We first investigate the influence of possible $k^{18}\text{O}$ changes, this is needed to establish precisely the evaporation regime prevailing in the source region for Antarctic precipitation. Based on calculation by Kangos²⁰, Friedman *et al.*²¹ suggest that this source region is south of 40 – 45°S in the Antarctic summer and south of 50 – 55°S in the Antarctic autumn. This is corroborated

Fig. 1 Oxygen isotope (a), deuterium excess $d = \delta\text{D} - 8\delta^{18}\text{O}$, with the smoothed profile and values of ice core increments represented by stars (b) and sodium marine contributions along the Dome C ice core. Panel d represents $d_{1s} = \delta\text{D} - 8.4\delta^{18}\text{O}$. Ages are estimated from a simple ice flow model assuming a variable rate of accumulation.

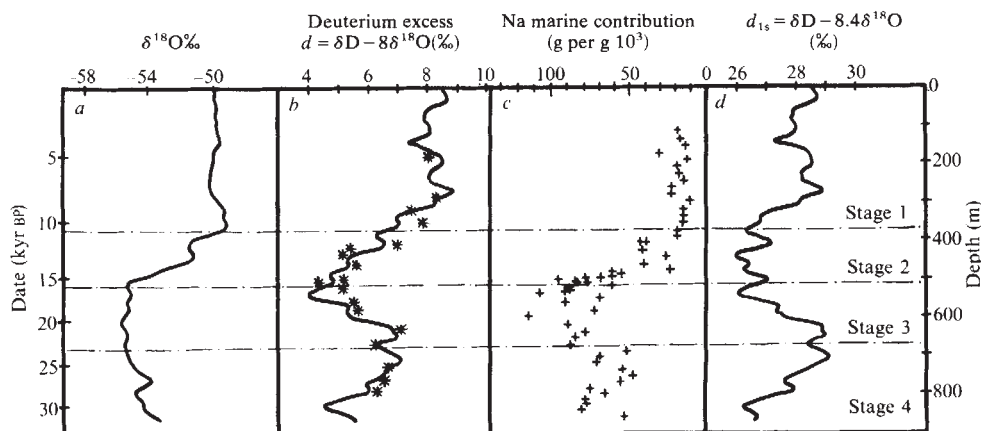


Table 1 Weighted mean of $k^{18}\text{O}$ (‰) for different minimum (U_m) and maximum (U_M) wind speeds (m s^{-1})

U_m	U_M	10	12	14	16	18	20
1		4.5	4.2	4.4	4.6	4.8	5.1
3		4.3	4.3	4.3	4.5	4.7	5.0
5		3.9	3.9	4.1	4.4	4.7	5.0

by the fact that the meridional flux of water vapour in the Southern Hemisphere has a maximum poleward value near 40°S (ref. 22) and by the suggestion that the zones between the subtropical convergence (average latitude about 41°S) and the ice edge should be taken as the source regions of maritime polar air²³.

Meridional profiles of the present day surface zonal winds exhibit large values south of 40°S . On annual scale, the hemispheric average presents a maximum value of $\sim 10\text{ m s}^{-1}$ between 50 and 55°S and a rapid decrease southward²⁴. These data refer to geostrophic winds which are generally fairly consistent with observed winds²⁴. On the other hand, both theoretical and experimental results now available show that atmospheric circulation was stronger during the glacial age, especially due to the Pole–Equator temperature gradient increase²⁵. Simulations of ice climate show acceleration of meridional circulation over oceanic regions²⁶ and intensification in high latitudes of the direct circulations involving sinking movements near the poles²⁷. Strong experimental evidence that they affect both ocean and land is provided by the Dome C ice core with large marine and continental inputs respectively 5 and 20 times higher than present at the end of the last glacial age²⁵. A wind speed increase of $5\text{--}8\text{ m s}^{-1}$ over continental regions was estimated²⁵. Curve 1c, showing the oceanic component of Na (the theoretical contribution was subtracted from Al measurements) may tentatively be explained²⁵ by stronger aerosol production which is mainly driven by the wind velocity at the sea surface^{28,29}, indicating stronger winds in the source region for Antarctic precipitation during the glacial maximum than at present.

As a result, whatever the period, a part of the water providing moisture for Antarctic precipitation is evaporated in rough regime conditions. To calculate the average d value of the first precipitation, it is therefore necessary to use a weighted value of $k^{18}\text{O}$. The evaporation flux is written¹⁷:

$$E = C_q \rho U q_s (1 - h) \quad (5)$$

where C_q , ρ , U and q_s are respectively the bulk transfer coefficient, the air density, the windspeed at height z and the saturated specific humidity at the water-surface temperature. The same reference level applies for h and U ($z = 10\text{ m}$). As a rough estimate of the average $k^{18}\text{O}$ over the source region, we have calculated

$$\int_{U_m}^{U_M} k_{18\text{O}} U dU / \int_{U_m}^{U_M} U dU$$

U_m and U_M being the minimum and maximum values of U . In this way, we neglect weighting by the specific humidity gradient, $q_s(1-h)$ and by the different areas involved, which appears justified for our present purpose which is limited to an evaluation of the $k^{18}\text{O}$ change between present and glacial conditions. The results are given in Table 1 as a function of U_m and U_M over ranges of values ($1\text{--}5\text{ m s}^{-1}$ for U_m and $10\text{--}20\text{ m s}^{-1}$ for U_M) which cover the present and probably glacial conditions. Very weak variations of $k^{18}\text{O}$ are observed for $10 < U_M < 16\text{ m s}^{-1}$ and a slight increase (around 1) is shown for U_M values of 18 and 20 m s^{-1} (when compared with $U_M = 10\text{ m s}^{-1}$). This higher $k^{18}\text{O}$ would result in a slightly higher d value (equation (3)) showing that the lower glacial d value at glacial maximum cannot be explained by the wind speed increase over the source region for Antarctic precipitation. Similarly, this low d value could not be assigned to a reduction of wind speed at glacial period which would lead to an increase in the relative import-

ance of the smooth regime and thus $k^{18}\text{O}$.

Anticipating that a modification of the $\delta D\text{--}\delta^{18}\text{O}$ slope does not largely influence the d values, it is seen from equation (4) that the d lowering of about 4.5‰ observed between the past 7,500 yr and 18,000 yr BP is attributable to a higher value of h . An h increase of 10% (calculated with a mean $k^{18}\text{O}$ of 4.5‰) is derived indicating that relative humidity over the oceanic surfaces was around 90% for full glacial conditions.

The first approach to determining the influence of a slope change was to calculate $d_{18} = \delta D - 8.4\delta^{18}\text{O}$, with 8.4 being the slope of the least squares line using all the samples. The smoothed curve presented on Fig. 1d shows that the d trend is not greatly affected, indicating that a different, but unique, slope value cannot explain the observed d changes. Another possibility is to assume that s varied with time. This second point can be discussed in two parts.

First, for liquid precipitation, s depends both on h and T_e (temperature of evaporation). Keeping T_e constant, a change of h from 0.8 to 0.9 would decrease s by 0.15 (ref. 7). However, CLIMAP project results¹¹ show that average August sea surface temperature south of 40°S was $\sim 2^\circ\text{C}$ lower 18,000 yr BP than at present, and in these conditions the slope reduction is only by 0.06. As this effect applies only to the first part of the water cycle for which $\delta^{18}\text{O}_{\text{pi}} - \delta^{18}\text{O}$ is $< 20\text{‰}$, the resulting Δd_i increase does not exceed 1.2‰.

Second, at snow formation, occurring in a supersaturated environment (over ice) a kinetic fractionation effect similar to that existing at evaporation has to be taken into account (J.J. and L.M., in preparation). The $\delta D\text{--}\delta^{18}\text{O}$ slope then increases with the value of supersaturation. It could be suggested that a decrease of supersaturation and thus of slope would result from the higher aerosol content at glacial periods; however, such a decrease would also cause an increase in d .

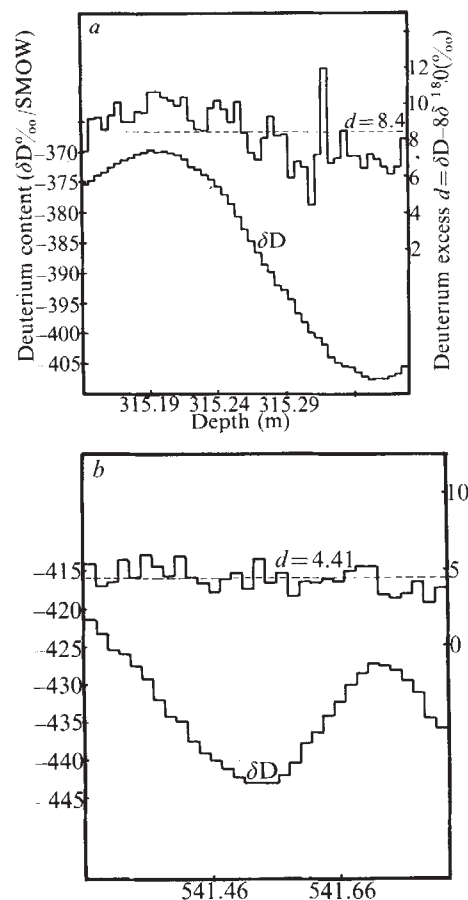


Fig. 2 Detailed deuterium and deuterium excess profiles in *a*, an ice core piece from the Holocene period; *b*, an ice core from the glacial maximum.

The possible changes both of $k^{18}\text{O}$ and s would therefore lead to higher d values at glacial periods. Anyway, the influence of variations of $k^{18}\text{O}$ and s for the first part of the water cycle is small. The effect of a more hypothetical slope change at snow formation was not estimated but if it exists the present interpretation of higher relative humidity would be also reinforced. This last point could be investigated from the comparison with d data obtained on other, existing or future, ice cores. (Note, however, that interpretation of data from more coastal Antarctic sites will be complicated by the change in snow origin.) More generally, it would be very interesting to compare the Dome C d profile with that in other ice cores both from Greenland and Antarctic ice sheets.

From energy balance considerations, $\sim 10\%$ less evaporation would be anticipated³⁰. Atmospheric modelling of ice age climate shows a comparable decrease²⁷. Taking into account the 2°C reduction of the sea-surface temperature¹¹, leading to an average reduction of q_s of $\sim 15\%$, equation (5) shows that a 10% decrease of evaporation, as suggested above, is obtained by keeping the $(1-h)U$ value practically unchanged. An h value of 90% (instead of 80%), as suggested by d changes, thus infers, on average, a doubling of the wind speed over all the oceanic area concerned. Although these are many assumptions in such an interpretation, it does point out the high consistency of two experimental results from the Dome C ice core at $18,000$ yr BP, that is, a lower d value and high Na (and also aerosols) content, indicating respectively a higher relative humidity close to the oceanic surface and a higher wind speed.

Remarkably, such a correlation between d and Na profiles exists all along the core (Fig. 1), tending to show that relative humidity and wind speed vary in a parallel manner with values

for stages 2 and 4 in between those prevailing during the Holocene and maximum glacial periods. The entire d profile clearly contains interesting information regarding the relative humidity changes over the past $32,000$ yr. For example, relatively low d values encountered around $4,000$ yr BP (Fig. 1b) coincide with the drier climates that have characterized the past $4,000$ – $4,500$ yr (ref. 31). This is in agreement with the fact that a d decrease probably reflects an h increase which means (neglecting the possible changes of U and q_s), a reduction of the evaporation rate (equation (5)). It is also observed that the wet phase ending $25,000$ yr BP in southern Australia presents higher d values than the following drier $25,000$ – $14,000$ yr BP period³². Dating uncertainties prevent detailed discussion of the synchronism between d and wetness changes but changes from wet to dry conditions are apparently accompanied by a d decrease and vice versa, which is quite consistent with our interpretation on d changes.

However, at its present stage of interpretation, the main result of this study is the strong indication of higher relative humidity over the ocean surface (estimated around 90%) south of 40°S during the late glacial maximum. A similar change probably also affected the tropical and equatorial regions as suggested either from isotopic data^{6,7} or from theoretical considerations³³. Such data have probably to be taken into account when developing climate modelling of the ice age.

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Signal recognition particle contains a 7S RNA essential for protein translocation across the endoplasmic reticulum

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In addition to its previously characterized, six different polypeptide components, signal recognition protein—which functions in protein translocation across and integration into the endoplasmic reticulum membrane—contains a 7S RNA molecule. The RNA is closely identified with the small cytoplasmic 7SL RNA and is required for both structural and functional properties of signal recognition protein—which we therefore rename signal recognition particle.

THE signal recognition protein (SRP) has been previously isolated as an 11S complex consisting of six distinct polypeptide chains¹ from a salt-wash of microsomal vesicles², and is an essential component in the process of protein translocation

across the lipid bilayer of the endoplasmic reticulum¹. SRP functions in decoding the information contained in the signal peptide of nascent secretory^{3–5}, lysosomal (A.H. Erickson, P.W. and G.B., in preparation) and certain membrane⁶ proteins to

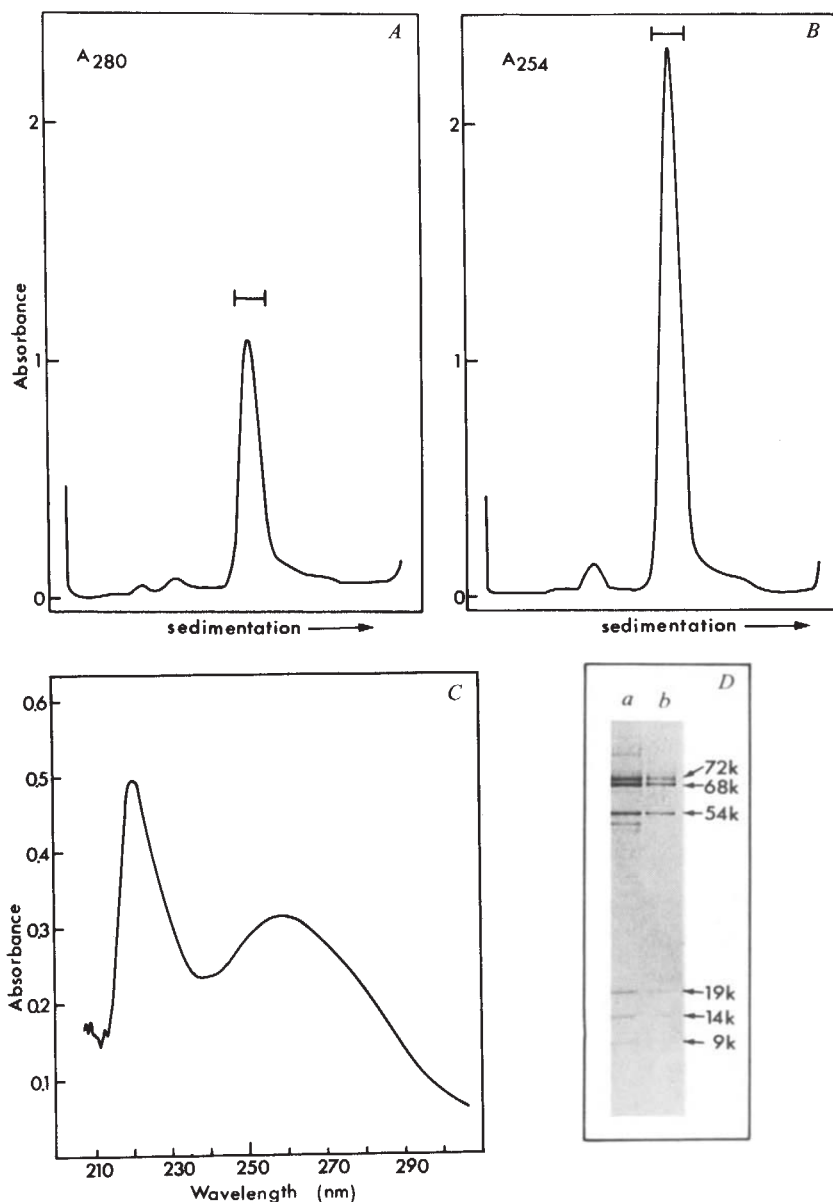


Fig. 1 Co-purification of SRP and SRP-RNA. SRP was extracted from 500 ml of dog pancreatic microsomes ($50 A_{280}$ units ml^{-1})³ and purified by chromatography on aminopentyl agarose as described previously¹. The eluent of the hydrophobic column was diluted with four volumes of water to reduce the salt concentration to 200 mM KOAc and passed over a 10-ml column of DEAE-Sephacrose 6B-Cl. The column was washed with 50 ml of a solution containing 50 mM triethanolamine/HOAc pH 7.5 (TEA), 350 mM KOAc, 3.5 mM $Mg(OAc)_2$, 1 mM dithiothreitol (DTT), 0.01% Nikkol (nonionic detergent from Nikko)¹ and eluted with a step of a solution containing 50 mM TEA, 600 mM KOAc, 6 mM $Mg(OAc)_2$, 1 mM DTT, 0.01% Nikkol. SRP eluted as a sharp peak (absorbance at 280 nm was monitored) and was collected as a 5-ml fraction ($3 A_{280}$ units ml^{-1}). Aliquots (0.5 ml) of this concentrated SRP solution were layered on top of 5–20% sucrose gradients (12 ml) containing 50 mM TEA, 500 mM KOAc, 5 mM $Mg(OAc)_2$, 1 mM DTT, 0.01% Nikkol and centrifuged for 20 h at 40,000 r.p.m. in the Beckman SW40 rotor as described previously¹. The gradients were fractionated using an ISCO gradient fractionator with a continuous flow cell (5 mm path length) and the absorbance was recorded at 280 nm (panel A) and 254 nm (panel B). Sedimentation was from left to right. The 11S peak fraction was collected as indicated by the vertical bar and used for all following experiments. It had a concentration of $1.1 A_{280}$ units ml^{-1} or $1.6 A_{260}$ units ml^{-1} and its activity was $20 U \mu l^{-1}$ (ref. 1). A 100- μl aliquot of the gradient purified SRP was diluted with 400 μl of water and an absorption spectrum was recorded using a Beckman DU-8 spectrophotometer with wavelength scanning accessory (panel C). A 30- μl fraction of the SRP preparation before (panel D, lane a) and after (panel D, lane b) sucrose gradient centrifugation was precipitated with trichloroacetic acid (TCA) and subjected to SDS-polyacrylamide gel electrophoresis on 10–15% gradient gels. Polypeptides were visualized by staining with Coomassie blue. The six SRP proteins¹ are indicated with their corresponding molecular weights.

the effect that it mediates the specific attachment of this class of polysomes to the microsomal membrane⁷. In the absence of microsomal membranes SRP specifically arrests the elongation of these proteins *in vitro*³ just after the signal peptide has emerged from the ribosome, thereby preventing the completion of pre-secretory proteins in the cytoplasm⁸. On interaction of these arrested ribosomes with a specific integral membrane protein—the SRP receptor^{9,10}—on the microsomal membrane, this elongation arrest is released and the nascent chain is translocated across⁸ or—as in the case of certain integral membrane proteins—integrated into⁶ the lipid bilayer.

Several small RNAs have been found and characterized in eukaryotic cells (for a recent review see ref. 11), but besides the tRNAs and the ribosomal 5S and 5.8S RNA, no physiological function could be directly demonstrated for any of them at the molecular level. The RNA of particular interest in the present study has been termed 7SL RNA (alternative nomenclatures are 7S scRNA¹² and ScL¹³) and it was first described as a component of some oncornaviruses¹⁴. It was later also shown to be a constitutive component of all uninfected cells¹⁵. 7SL RNA was usually obtained in cytoplasmic cell fractions, where it was found associated with polysomes or microsomal membranes^{12,13,16}, although some investigators also detected it in nuclear fractions¹⁷. It is a relatively abundant small RNA

which has been highly conserved through evolution^{12,15,17} and it was recently sequenced by two independent groups from either a cDNA clone of the human RNA^{18,19} or by direct RNA sequencing of the rat RNA²⁰. Both sequences are essentially identical and confirmed a previous observation²¹ that long stretches of 7SL RNA are homologous to the Alu family DNA sequences. Thus, the ~300-nucleotide long 7SL RNA was shown for about 100 nucleotides at its 5' end and ~50 nucleotides at its 3' end^{18,20} to have about 80% homology with the Alu consensus sequence²². The core portion of 7SL RNA (~150 nucleotides) showed no homology to Alu DNA, and is complementary to a new family of repeated DNA (but repeated at a lower frequency than Alu DNA) in the genome¹⁸.

We demonstrate here that 7SL RNA is part of a ribonucleoprotein (RNP). This RNP is identical to the previously purified and characterized signal recognition protein¹, the name of which we consequently change to signal recognition particle (SRP). We show that the RNA is indispensable for SRP's function. Thus, 7SL RNA has a function in the process of protein translocation across the endoplasmic reticulum.

SRP contains a RNA moiety

In our studies on SRP we noticed a relatively high ratio of the absorbances at 260 to absorbance at 280 nm in our SRP prepar-

ations isolated by hydrophobic chromatography¹. To determine whether this property was truly associated with SRP, we further purified SRP by DEAE chromatography and sucrose gradient centrifugation¹. Analysis of the sucrose gradients revealed a higher absorbance at 254 nm (Fig. 1B) than at 280 nm (Fig. 1A) in the 11S peak fraction. The 11S peak was collected (as indicated by the horizontal bars in Fig. 1A, B) and an absorption spectrum was recorded. From the spectrum (Fig. 1C) it was apparent that SRP had a relative absorption maximum at 258.5 nm. This finding strongly suggested that SRP contained a nucleic acid component which co-purified with the six polypeptide chains previously identified as protein components of SRP¹ (compare the polypeptide profile of the material before and after sucrose gradient centrifugation Fig. 1D, lanes *a* and *b*). As most of the absorbance at 260 nm could be rendered cold acid-soluble by base hydrolysis (see Table 1), we concluded that SRP contained a RNA moiety. We will refer to this RNA as SRP-RNA.

The RNA is required for SRP function

To demonstrate the involvement of SRP-RNA in the functions of SRP, we used *in vitro* assays for the SRP-dependent translocation of secretory proteins across microsomal membranes. Two principal activities of SRP can be distinguished in these assays.

First, SRP restores the ability of salt-extracted microsomes to translocate nascent secretory proteins^{1,7}. If a mixture of bovine pituitary mRNA and rabbit reticulocyte mRNA was translated in the wheat germ cell-free system in the presence of salt-extracted microsomes, preprolactin and globin were synthesized (Fig. 2A, lane *a*). Only when the system was supplemented with purified SRP did translocation and processing of the secretory protein take place (note the disappearance of preprolactin and the appearance of prolactin in Fig. 2A, lane *b*).

Our second assay for SRP activity is based on the observation that SRP in the absence of microsomal membranes specifically inhibits the translation of secretory but not of cytoplasmic proteins³. The presence of SRP in a translation system devoid of microsomal membranes therefore caused a severe decrease in the amount of preprolactin synthesized (Fig. 2D, compare lanes *a* and *b*), but did not affect the amount of globin synthesized.

To test whether SRP-RNA is a constitutive and indispensable part of SRP, we treated SRP with micrococcal nuclease. As this nuclease is Ca^{2+} dependent, it can be conveniently inactivated by the addition of Ca^{2+} -chelators²³. A rapid decrease of the translocation activity (Fig. 2C) and of the elongation-arresting activity (Fig. 2F) was observed with increasing digestion time, when compared with the control values of an incubation

Fig. 2 SRP-RNA is required for SRP's function. Bovine pituitary and rabbit reticulocyte RNA were translated together in a wheat germ cell-free system (25 μl) either in the presence (panels A–C) or in the absence (panels D–F) of 1 equivalent of salt-extracted microsomes as described previously³. In panels A, B, D and E translations were supplemented with 10 U (ref. 3) of gradient-purified SRP (see Fig. 1) where indicated (SRP+). In panels B and E the translations also contained 600 ng of SRP-RNA (see Fig. 4) where indicated (SRP-RNA+). Translation products were displayed by SDS-polyacrylamide gel electrophoresis and visualized by autoradiography as shown in panels A and D. Bands corresponding to preprolactin (pPL), prolactin (PL) and globin (GLO) are indicated. The minor band between pPL and PL is pregrowth hormone. Its processed form (growth hormone) migrates as a band below PL (panel A, lane *b*). For quantitative interpretation, the bands corresponding to pPL and PL were excised from the gel and their radioactivity determined as described previously³⁴. From the measurements obtained, per cent processing³ (panels B, C) and per cent inhibition⁶ (panels E, F) were calculated. In panels C and F, SRP was digested with *Staphylococcus aureus* nuclease (Boehringer) before it was used to supplement the translation systems. Fractions (10 μl) of gradient-purified SRP (see Fig. 1) were diluted on ice with 40 μl of a digestion mix to yield final concentrations of 1 mM CaCl_2 and 0 U ml^{-1} (circles), 150 U ml^{-1} (squares) or 750 U ml^{-1} (triangles) nuclease, respectively. Incubation was carried out at 30 °C. After 0, 10 and 60 min of digestion, aliquots (10 μl) were withdrawn and the nuclease was inactivated by the addition of 1 μl 20 mM EGTA. A 3- μl aliquot from each time point was assayed for SRP activity in 25 μl translations and the results quantitated as described above. Background (–SRP) and control (+SRP) values can be taken from the data in panels B and E (lanes *a* and *c*, respectively) because the data were plotted on a comparable scale.

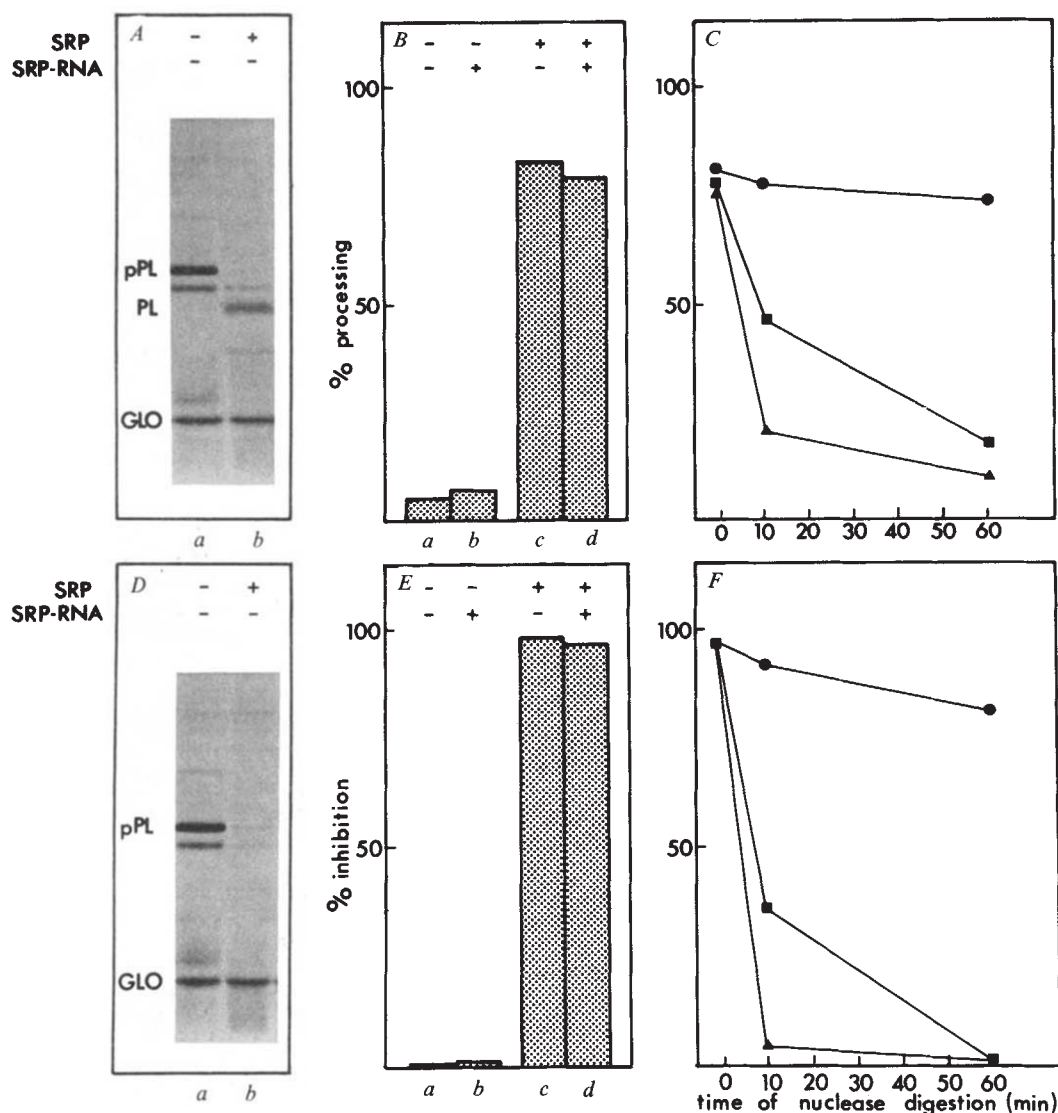
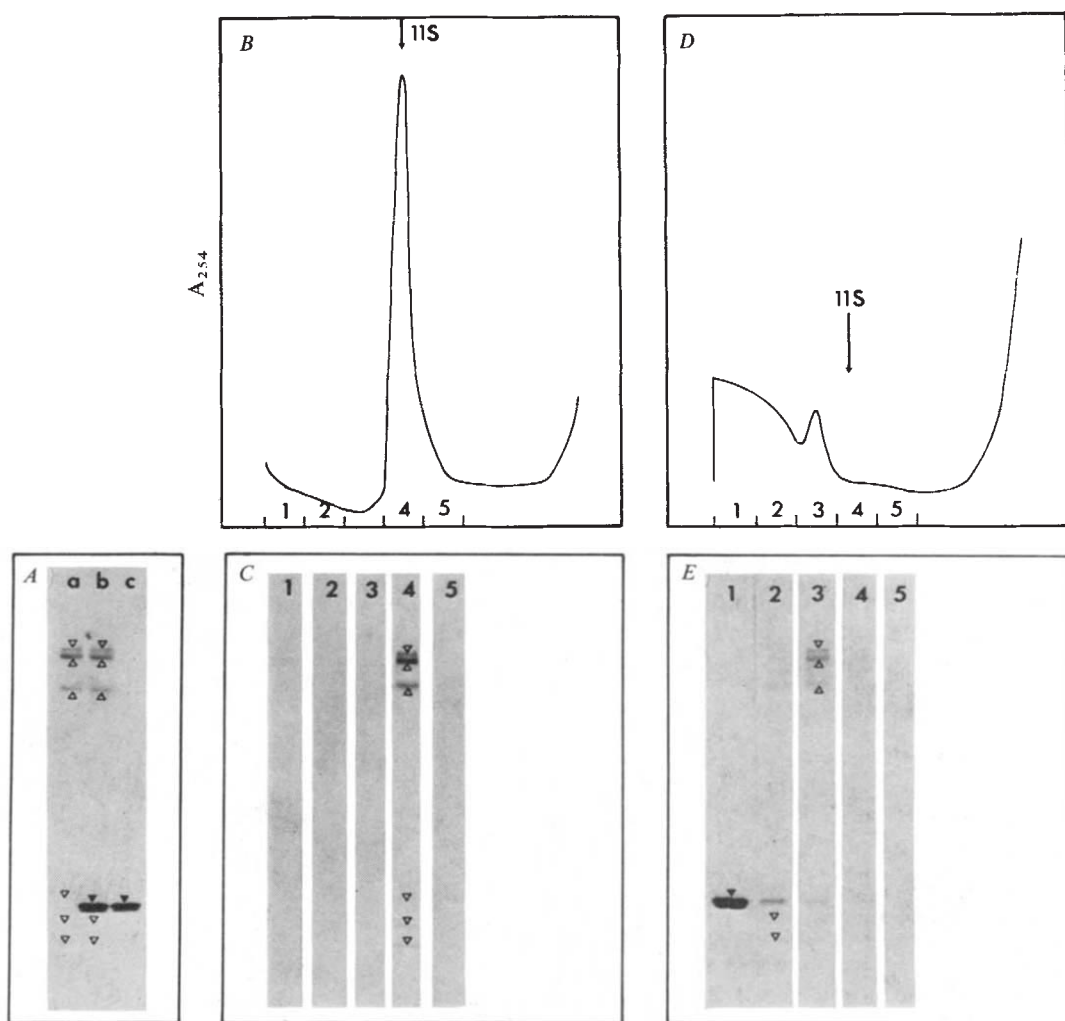


Fig. 3 SRP proteins change their sedimentation behaviour on nuclease digestion. Aliquots of gradient-purified SRP (see Fig. 1) were incubated in the absence or presence of 750 U ml⁻¹ of nuclease as described in Fig. 2 legend, except that the reactions were scaled up 10-fold. After incubation, an aliquot (100 µl) of each reaction mixture (panel A, lane a (no nuclease), lane b (750 U ml⁻¹ nuclease)) and 75 U nuclease (panel A, lane c) was TCA precipitated and the polypeptides displayed by SDS-polyacrylamide gel electrophoresis and visualized by Coomassie blue staining. Another aliquot (250 µl) of the reaction mixture was layered on top of a 5–20% sucrose gradient (5 ml) in 50 mM TEA, 500 mM KOAc, 2 mM EDTA, 0.01% Nikkol and centrifuged for 6 h at 50,000 r.p.m. in a Beckman SW50.1 rotor. Gradients were fractionated using an ISCO gradient fractionator. Absorbance was monitored at 254 nm (panel B, no nuclease; panel D, 750 U ml⁻¹ nuclease), five fractions were collected as indicated, TCA precipitated and the polypeptides displayed by SDS-polyacrylamide gel electrophoresis and Coomassie blue staining (panels C, E). Bands corresponding to the six SRP proteins are marked with open arrow heads, bands contributed by the nuclease (see panel A, lane c) are marked with solid arrow heads.



where the nuclease was omitted. The effect is concentration dependent with respect to nuclease, because higher nuclease concentrations lead to a more rapid inactivation of SRP. Note, however, that to see a marked effect these digestion conditions had to be relatively harsh (for example, 150 and 750 U ml⁻¹ nuclease for 10–60 min at 30 °C) compared with the nuclease treatment routinely given to microsomal membrane fractions or translation systems to deplete them of endogenous mRNA activity (20 U ml⁻¹ nuclease for 5 min at 25 °C). It is therefore not surprising that no loss of translocation activity of microsomal membranes was detected previously on mild nuclease treatment^{2,24}.

Analysis of the polypeptide profile of SRP before and after nuclease treatment (Fig. 3A, lanes a, b) revealed that the electrophoretic mobility of five of the six polypeptides was not altered (the sixth was buried under a band contributed by the nuclease and could therefore not be analysed). Taken together with the fact that SRP incubated with nuclease but in the presence of both Ca²⁺ and EGTA was not inactivated (data not shown), the explanation that the loss of SRP activity on nuclease digestion was due to a protease contamination can be ruled out.

RNase treatment changes the sedimentation behaviour of the SRP proteins

When nuclease-treated SRP was analysed by sucrose gradient centrifugation, the sedimentation characteristics of the SRP proteins appeared changed. Whereas SRP incubated in the

absence of nuclease sedimented at 11S (Fig. 3B, C) as previously described (ref. 1 and Fig. 1), on extensive nuclease digestion the sedimentation of the SRP proteins was retarded, although they still did not sediment as monomeric species (Fig. 3D, E). Interestingly, two subfractions seemed to be resolved, one containing the three high molecular weight proteins sedimenting at about 8S and one containing two (possibly all

Table 1 Stoichiometry between protein and RNA components in SRP

	RNA (µg per 100 µg SRP)	Protein
Assay	21	79
Calculated	27	73

For the assay, gradient-purified SRP was treated with 0.3 M KOH for 1 h at 60 °C. After precipitation with cold HClO₄ the RNA content of SRP was calculated from the absorbance at 260 nm of the supernatant fraction³². Absorbance measurements were converted into µg RNA by assuming an average nucleotide molecular weight of 330 and an average nucleotide molar extinction coefficient of 10⁴ at 260 nm. Protein in the SRP fraction was determined according to Schaffner and Weissman³³ using bovine serum albumin as a standard. According to these measurements our SRP preparation (20 U µl⁻¹ (ref. 1), see Fig. 1) contained 75 µg ml⁻¹ RNA and 285 µg ml⁻¹ protein. The proportions were calculated based on our size estimations of SRP-RNA (260 nucleotides) and the molecular weights of the six SRP proteins as estimated by SDS-polyacrylamide gel electrophoresis (see ref. 1 and Fig. 1). SRP was assumed to consist of one molecule of each of these components.

three, see above) of the low molecular weight proteins sedimenting at about 5S.

Isolated SRP-RNA has no measurable activity

It was previously reported that the isolated RNA of a cytoplasmic RNP fraction possessed the same translation inhibitory activity as the intact particles²⁵. To test whether isolated SRP-RNA would exhibit a direct effect in our assay systems, SRP-RNA was extracted from a gradient-purified SRP preparation. It was then determined whether it would exhibit either a SRP-like activity by itself or whether it could be shown to compete with intact SRP. As shown by the data in Fig. 2B and E, the isolated SRP-RNA (even in eightfold higher molar concentrations than a saturating amount of SRP) neither restored translocation competence to salt-extracted microsomes (Fig. 2B, compare lanes *a* and *b*) nor inhibited translation of prolactin mRNA (Fig. 2E, compare lanes *a* and *b*) or globin mRNA (data not shown). When SRP was assayed in the presence of an eightfold molar excess of SRP-RNA, the SRP-RNA also did not compete with SRP in either of the assays (Fig. 2B, E, lanes *c*, *d*).

Characterization of the SRP-RNA

From the above experiments we conclude that the RNA moiety is a constitutive part of SRP in a structural as well as functional

sense. We therefore attempted to characterize this SRP-RNA more carefully. When the extracted RNA was electrophoresed on a polyacrylamide gel in denaturing conditions, one major and one minor band could be resolved on ethidium bromide staining (Fig. 4A). From the staining intensity we estimated that the major band (slower mobility) was comprised of about four times more RNA than the lower molecular weight band.

Having established that SRP-RNA appeared to be of a discrete size(s), we attempted to label the molecules at the 3' end using T₄ RNA ligase and 5'-³²P-pCp. The pattern of the 3'-labelled SRP-RNA obtained after autoradiography of a polyacrylamide gel (Fig. 4B) was identical (qualitatively as well as quantitatively) to that obtained after ethidium bromide staining (compare with Fig. 4A). We also labelled a small nuclear RNA (snRNA) fraction from HeLa cells (a gift of Dr D. Adams) with 5'-³²P-pCp and used it as markers in this electrophoresis system (Fig. 4C). From the sizes of the snRNAs¹¹, we extrapolated the size of the SRP-RNA to be about 260 nucleotides and 245 nucleotides for the major and the minor molecular weight species, respectively (Fig. 4D).

Relationship of SRP-RNA to 7SL RNA

To determine the possible relationship of SRP-RNA to any known RNA sequences, we performed partial sequence analysis. The 260-nucleotide and 245-nucleotide bands of 3'-labelled

Fig. 4 Size characterization of SRP-RNA. RNA was extracted by the EtOH/perchlorate procedure³⁵ after proteinase K treatment of gradient purified SRP. First we concentrated SRP further by EtOH precipitation. To an 800- μ l fraction of gradient-purified SRP we added 2 ml of EtOH. The mixture was kept for 30 min at -80°C and SRP was pelleted by centrifugation for 15 min at 10,000 g_{av} . The wet pellet was dissolved in 120 μ l of a solution containing 25 mM TEA, 2.4% SDS, 100 mM NaCl, 15 mM EDTA and 200 $\mu\text{g ml}^{-1}$ proteinase K (Boehringer). The mixture was incubated at 37°C for 30 min, then 80 μ l of a solution of 3.5 M NaClO₄ was added and the mixture heated to 50°C for 5 min. A saturated solution (800 μ l) of NaClO₄ in EtOH/water (80:20) was added, the mixture was incubated for 45 min on ice and the RNA was pelleted for 15 min at 10,000 g_{av} . The pellet was taken up in 50- μ l sterile 300 mM NaOAc, 1 mM EDTA and reprecipitated with 125 μ l of EtOH after a 30-min incubation at -80°C . This step was performed twice more. The final pellet was rinsed with 100 μ l ice-cold EtOH/water (80:20), lyophilized to dryness and taken up in sterile water. The yield was 60 μg of RNA based on absorbance at 260 nm ($20 A_{260}$ units = 1 mg). SRP-RNA was labelled at the 3' end following the procedure of England *et al.*³⁶. A sample of 0.4 μg RNA was incubated with 25 pmol 5'-³²P-pCp (NEN) and 500 U ml^{-1} T₄-RNA ligase (PL Biochemicals) for 8 h at 4°C . Carrier tRNA (6 μg , Boehringer, calf liver) was added and the RNA reisolated by EtOH/perchlorate precipitation, followed by EtOH precipitation and a final rinse with 80% EtOH. 2% Of the label was incorporated into SRP-RNA. A sample of 0.4 μg snRNA from HeLa cells was labelled by the same procedure. The isolated SRP-RNA (2 μg , lane A), the 3'-labelled SRP-RNA (1,000 d.p.m., lane B) and the 3'-labelled HeLa snRNA (10,000 d.p.m.) were denatured in 50% formamide in 50 mM Tris, 50 mM boric acid, 2 mM EDTA (TBE) for 4 min at 50°C and electrophoresed in a 50-cm long, 0.4-mm thick 10% polyacrylamide gel containing 50 mM TBE and 7 M urea at 1,000 V until the xylene cyanole marker dye was 10 cm from the bottom. The gel was stained in 1 $\mu\text{g ml}^{-1}$ ethidium bromide for 30 min, destained for 30 min in water, photographed wet under UV light (lane A) and dried. Radioactive bands were visualized by autoradiography (lanes B, C). The HeLa snRNAs were assigned according to ref. 37 and their sizes¹¹ were taken to be: U3, 216 nucleotides (NT); U2, 196 NT; U1, 171 NT; 5.8S, 158 NT; U4, 147 NT; 5.0S, 121 NT; U5, 114 NT. The standard curve (panel D) was constructed by plotting the square root of the number of nucleotides over the logarithm of the relative mobility³⁸ and fitting a straight line by the least-square method.

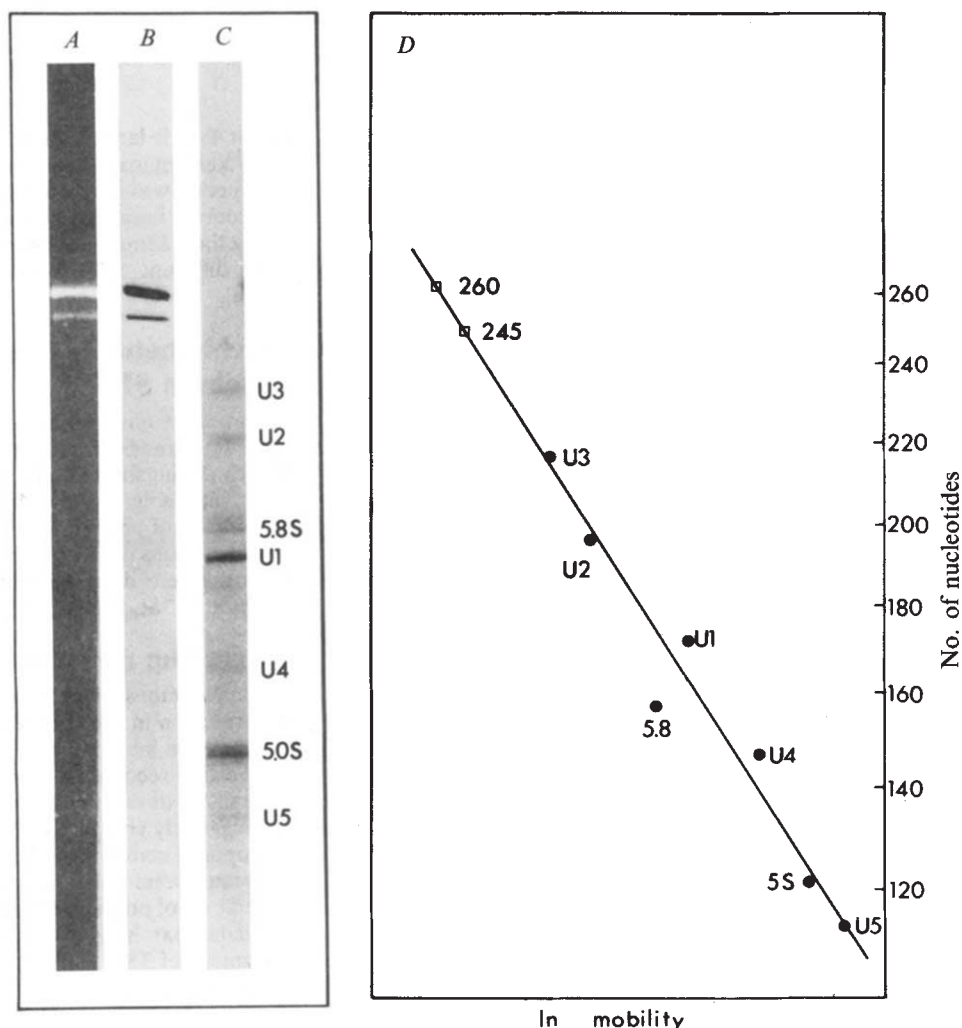
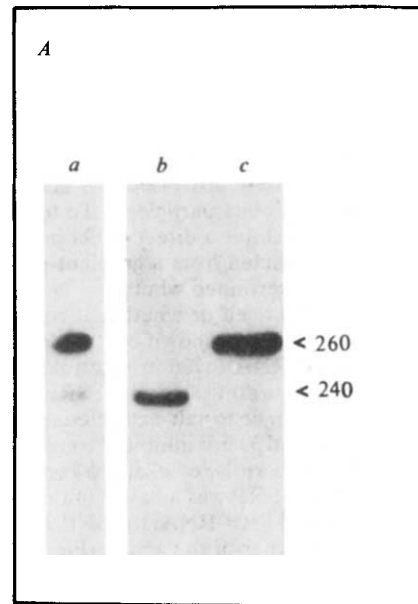


Fig. 5 (Right and opposite page.) Partial sequence determination of SRP-RNA. The 260- and the 245-nucleotide long 3'-labelled SRP-RNAs were separated by polyacrylamide gel electrophoresis²⁸ as described in Fig. 4 legend except that the gel was 7%, 30 cm long and 1.2 mm thick (panel A, lane a). Bands were localized by autoradiography of the wet gel, excised with a sterile scalpel, each rinsed for 30 min with 1 ml of sterile water and eluted on an overhead shaker overnight at 37 °C into 1 ml of sterile 0.5 M NH₄OAc, 1 mM EDTA, 10 µg ml⁻¹ tRNA. The eluate was filtered through a 0.22-µm Millipore filter, EtOH precipitated and reprecipitated from 100 µl 300 mM NaOAc, 1 mM EDTA with 250 µl EtOH. The pellet was rinsed with 100 µl 80% EtOH and lyophilized. Aliquots were re-electrophoresed in the conditions described above and showed no breakdown or cross-contamination of the two species (panel A, lanes b, c). Chemical modification reactions followed by aniline strand cleavage were performed as described by Peattie²⁶ with the following modifications: the 'A reaction' contained 2 µl diethylpyrocarbonate and 10 µl EtOH; incubation was for 10 min. The 'G, C and U reactions' were incubated for 1, 20 and 12 min, respectively. The cleaved RNA was denatured in 50% formamide, 50 mM TBE and electrophoresed on a 40-cm long, 0.4-mm thick 20% polyacrylamide gel in 100 mM TBE (acrylamide to bisacrylamide in all sequencing gels was 20:1) in a 37 °C incubator at 1,000 V until the bromophenol blue marker dye was 20 cm from the bottom. The autoradiogram of the wet gel is shown in panel B. 10,000 d.p.m. were loaded per lane. Cleavage products of the 260-nucleotide SRP-RNA of the C, U, G and A-reaction are shown in lanes 1, 2, 3 and 4, respectively. Cleavage products of the 245-nucleotide SRP-RNA of the A reaction are shown in lane 5. The sequence assignments are indicated by dots on the bands. The numbering of the assigned residues is according to Ullu *et al.*¹⁸ (see also Fig. 6). The position of the marker dyes bromophenol blue (BPB) and xylene cyanole (XC) is indicated. On parallel lanes (not shown) cleavage products of 3'-labelled 5S RNA of *Bombyx mori* were electrophoresed to confirm the fidelity of the sequencing reactions on the basis of its known sequence³⁹. The arrow at the bottom indicates the position of the 3'-terminal U residue of this 5S RNA. Its larger fragments migrated in the same positions as the SRP-RNA cleavage products. Residue 284 of SRP-RNA could not be unambiguously assigned. We used enzymatic sequencing reactions to determine a partial sequence further into the molecule. The reactions using U₂ RNase (cleaves after A residues) and T₁ RNase (cleaves after G residues) were carried out according to Donis-Keller *et al.*²⁷ in 7 M urea at 50 °C, except that the reaction volumes were scaled down to 10 µl and the carrier tRNA was at 100 µg ml⁻¹. We also performed a partial base hydrolysis²⁷ (cleaves the RNA randomly) and a control incubation in the presence of urea at elevated temperature without the addition of nuclease. Cleavage products were displayed by electrophoresis in an 8% polyacrylamide gel as described above and visualized by autoradiography of the dried gel. Electrophoresis was terminated after the xylene cyanole marker dye had moved 25 cm (panel C) and 50 cm (panel D) through the gel. Panel C: Lane 1, chemical A cleavage; lane 2, enzymatic cleavage with U₂ at 10 U ml⁻¹; lane 3, enzymatic cleavage with U₂ at 3 U ml⁻¹; lane 4, base ladder; lane 5, enzymatic cleavage with T₁ at 0.01 U ml⁻¹; lane 6, enzymatic cleavage with T₁ at 0.003 U ml⁻¹; lane 7, control (incubation in the absence of nuclease). Panel D: Lane 1, enzymatic cleavage with U₂ at 10 U ml⁻¹; lane 2, base ladder; lane 3, enzymatic cleavage with T₁ at 0.003 U ml⁻¹; lane 4, control (incubation in the absence of nuclease). The assignment of A and G residues is indicated by dots; Y indicates pyrimidine residues (C or U, which cannot be distinguished by this method), N indicates residues which could not be assigned because of ambiguous cleavage reactions or major fragments in the control lane. In panel D the base ladder bands were compressed and no sequence could be read between residues 188 and 191.



SRP-RNA were separated by preparative polyacrylamide electrophoresis and eluted from the gel (Fig. 5A, lane a). On re-electrophoresis they migrate as distinct bands of unchanged mobility (Fig. 5A, lanes b, c) and therefore appear not to be interconvertible conformational isomers of each other. We then subjected the 260-nucleotide SRP-RNA species to sequence analysis by either a complete set of chemical²⁶ (Fig. 5B) or a partial set of enzymatic²⁷ (Fig. 5C) cleavage reactions followed by polyacrylamide gel electrophoresis of the generated fragments²⁸ and autoradiography. The cleavage pattern allowed us to read the complete sequence of the SRP-RNA for 40 residues from the 3' end (Fig. 5B) and a partial sequence (only the A and G residues) for about 150 residues from the 3' end (Fig. 5C).

When we compared our partial sequence data (Fig. 6, line c) with the recently published sequences for 7SL RNA from man¹⁸ and rat²⁰ (Fig. 6, lines a, b), we found a perfect match in the sequence alignments (Fig. 6). Furthermore, our partial sequence is distinct from 7SK RNA (ref. 19 and E. Ullu, personal communication), the only other major small cytoplasmic RNA in the size class of interest. Both HeLa cell 7SL RNA and the 260-nucleotide SRP-RNA have an identical electrophoretic mobility in polyacrylamide gels (E. Ullu, personal communication) and we therefore attribute the apparent size difference between our estimation (260 nucleotides) and the published sizes for 7SL RNA [303 (ref. 18) and 295 (ref. 20) nucleotides] to either an anomalous behaviour of the RNA on electrophoresis or an inadequacy in our size extrapolation. We conclude that SRP-RNA is identical to 7SL RNA, although minor differences at the 5' end between the published forms of 7SL RNA and SRP-RNA cannot be ruled out.

We also subjected the 245-nucleotide long SRP-RNA species to a partial chemical sequencing reaction (allowing us to read the A residues). For the last 40 residues on the 3' end we obtained an identical cleavage pattern for the 260-nucleotide (Fig. 5B, lane 4) and the 245-nucleotide (Fig. 5B, lane 5) SRP-RNA, indicating that both bands have an identical 3'-terminal sequence. This identity in the cleavage pattern could be followed from the 3' end through the entire molecule if the fragments were displayed on lower percentage polyacrylamide gels (data not shown). We therefore conclude that the 245-nucleotide species was identical to the 260-nucleotide species

except that it lacked about 15 nucleotides at its 5' end. The most likely interpretation for this finding is that the 245-nucleotide species was derived from the 260-nucleotide molecule as a nucleolytic breakdown product. This interpretation is supported by the finding that the ratio of the two bands was variable when different SRP preparations were analysed (data not shown).

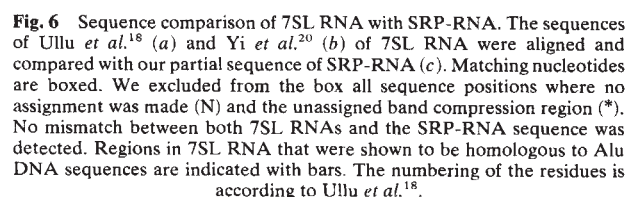
Stoichiometry between RNA and protein in SRP

We have previously estimated from the Coomassie blue staining intensity of the SRP proteins that SRP contains one molecule of each of the six different polypeptide chains¹. The data displayed in Table 1 show that the experimentally determined proportions of protein to RNA correspond reasonably well to the proportions calculated from our molecular weight determinations. These data therefore suggest that there is also one molecule of 7SL RNA per six polypeptide chains in SRP.

Concluding remarks

We have demonstrated here that 7SL small cytoplasmic RNA has a function in the early step of protein secretion and membrane protein insertion as a constitutive and indispensable part of the signal recognition particle. SRP was previously isolated by means of its distinct interaction with hydrophobic matrixes¹ as a negatively charged, stable particle containing six proteins. Its proposed complex mode of transient interactions⁹ with the membrane-bound SRP receptor⁹⁻¹¹, with ribosomes³, and with a specific set of polysomes³ might explain some of the contradictory data that had accumulated concerning the subcellular localization of 7SL RNA^{13,14,16-18}. Although we do not know whether all 7SL RNA is found in the cell as SRP, the availability of specific antibodies against three of the SRP proteins (P.W. and G.B., in preparation) should allow us to answer this question conclusively.

It is not clear what function 7SL RNA serves in SRP, but two different modes of action could be envisaged: (1) the RNA could have a merely structural role and could serve as a 'core' or 'matrix' on which the SRP proteins (or perhaps other accessory proteins like the SRP receptor or ribosomal proteins



(see below) RNP structure (SRP), the provocative possibility arises that Alu-like sequences could have arisen by re-iteration from SRP-RNA (rather than the unique core of SRP-RNA having picked up Alu-like sequences from the genome).

Both the 7SL RNA and the mode of co-translational protein translocation seem to be highly conserved through evolution³¹. We have recently demonstrated that the prokaryotic secretory protein β -lactamase, when translated on plant ribosomes (wheat germ), is translocated and correctly processed by mammalian microsomal membranes⁵. This translocation process was SRP dependent and the synthesis of the prokaryotic secretory protein could be arrested by SRP in the absence of microsomal membranes. These findings strongly suggest that the mechanism of co-translational protein translocation has been conserved and encourage our search for a prokaryotic counterpart of SRP and its small RNA. Thus, SRP seems to be an integral and

indispensable component of the protein synthesis machinery, ensuring the correct topogenesis of a specific subset of proteins. Considering its structural features and its intimate (although probably transient) functional association with ribosomes, it could almost be regarded as a 'third ribosomal subunit' functioning as the adapter between the cytoplasmic translation and the membrane-bound protein translocation machinery.

We thank D. Adams and M. Walberg for their advice and help concerning the RNA sequencing technology, P. Lizardi, H. Robertson, J. Steitz and A. Weiner for many helpful discussions and suggestions, and E. Ullu for making the sequence of 7SL RNA available to us prior to its publication. These studies were supported by NIH research grant GM-27155. P.W. is supported by a research fellowship from the Deutsche Forschungsgemeinschaft (Wa 478/1-1). Original prints of Fig. 5 are available on request from the authors.

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Mitochondrial and chloroplast genomes of maize have a 12-kilobase DNA sequence in common

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A 12-kilobase DNA sequence has been identified in the maize mitochondrial genome which is homologous to part of the inverted repeat of the maize chloroplast genome. In chloroplasts the sequence contains a 16S rRNA gene, and also the coding sequences for tRNA^{Ile} and tRNA^{Val}. Mitochondrial DNA from the male-sterile cytoplasms of maize is altered in this region.

INTERACTIONS between the nucleus and cytoplasmic organelles have a major role in eukaryotic cellular metabolism. Mitochondrial and chloroplast ATPases and some cytochromes, for example the yeast cytochrome *c* oxidase complex, are assembled post-translationally in the organelles from both nuclear and organelle encoded polypeptide subunits¹⁻⁹.

Although eukaryotic cells depend on the interactions of organellar gene products, the genetic material of the nucleus, mitochondrion and chloroplast is usually thought of as being maintained discretely in each organelle without duplication elsewhere. We report here, however, the characterization of a 12-kilobase (kb) DNA sequence that is found in both the chloroplast and mitochondrial genomes of maize. The similarity of the sequences, with more than 90% base sequence homology, is striking. Furthermore, this particular region of the mitochondrial genome is altered in three male-sterile lines of maize, suggesting that the maintenance of homology may be essential to normal plant development.

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Cross-hybridization between mitochondrial and chloroplast DNAs

³²P-labelled preparations of maize mitochondrial RNA (mtRNA) were found to hybridize weakly to two mtDNA *Sst*II restriction fragments of 1.75 and 1.58 kb (Fig. 1a), which were known not to contain the mitochondrial ribosomal RNA (rRNA) genes. Cosmid clones of mtDNA containing these restriction fragments, for example 83H4 (see Fig. 3e), hybridized weakly to the mitochondrial 18S rRNA, but much more strongly to an RNA molecule of ~1,500 nucleotides (Fig. 1b). As this is the size of the chloroplast 16S rRNA, the presence of which is demonstrated when total RNA is probed with a cloned wheat chloroplast 16S rDNA sequence¹⁰, it seemed likely that the mtRNA preparations used to probe the *Sst*II mtDNA digests were contaminated with chloroplast RNA, and that some sequence homology existed between the 1.58- and 1.75-kb mitochondrial *Sst*II fragments and the chloroplast 16S rRNA.

Restriction endonuclease digests of mtDNA were therefore probed with nick-translated maize chloroplast DNA (ctDNA)

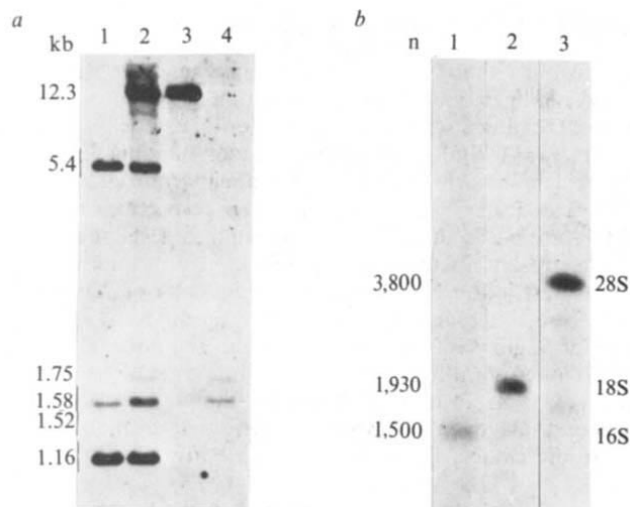


Fig. 1 Cross-hybridization between mtDNA and chloroplast rRNA. *a*, Mitochondrial ribosomal DNA cosmid clones³³ digested with *Ssr*II and electrophoresed in a 1% agarose gel, transferred to nitrocellulose³⁴ (S&S, BA85) and probed with 5' ³²P-labelled total RNA³⁵ isolated from purified mitochondria³⁶. (1) 2c21 which contains the 18S and 5S rRNA genes in the 5.4-, 1.52- and 1.16-kb fragments³⁷. (2) *Ssr*II digested mtDNA, (3) 2c13, which contains the 26S rRNA gene in a 12.3-kb fragment. (4) 83H4, although the cosmid does not contain mitochondrial rRNA genes, 5'-labelled RNA preparations hybridize weakly to 1.58- and 1.75-kb *Ssr*II fragments (see text and Fig. 1*b*, track 1). *b*, Cosmids 83H4, 2c21 and 2c13 were labelled by nick translation^{38,39}, and hybridized to Northern blots⁴⁰ of RNA isolated from coleoptiles³⁷ and electrophoresed in 1% agarose-6% formaldehyde gels⁴¹. (1) 83H4 hybridizes to a transcript which has the same electrophoretic mobility as chloroplast 16S rRNA. (2) 2c21 hybridizes predominantly to the 1,900-nucleotide transcript, and also weakly to the chloroplast 16S rRNA. (3) 2c13 hybridizes to a 3,800-nucleotide transcript (26S rRNA). The minor hybridizations to smaller transcripts are probably specific breakdown products of 26S rRNA³⁶. n, No. of nucleotides.

to determine the level of ctDNA contamination of mtDNA preparations. Little contamination was evident. Surprisingly, however, several mitochondrial *Bam*HI restriction fragments showed strong homology to the ctDNA probe and exhibited a similar electrophoretic mobility to some ctDNA *Bam*HI restriction fragments (Fig. 2*c*, tracks 1, 4). Similarly the mtDNA cosmid clones, which contained the 1.75-kb and 1.58-kb *Ssr*II fragments, when digested with *Bam*HI and probed with nick-translated chloroplast DNA, were found to contain all but one of the mitochondrial *Bam*HI fragments which hybridized to ctDNA (Fig. 2*c*, tracks 3, 4).

Physical mapping of the homologous region

As the *Ssr*II restriction map of this region of the mitochondrial genome was already known (Lonsdale and Hodge, unpublished), cosmids 91D10, 83H4 and 2c87, which span the region of the mitochondrial genome containing the homology to ctDNA were selected. The cosmids were nick translated and used to probe *Bam*HI digests of mtDNA, ctDNA and the cosmid DNAs (Fig. 2).

The cosmid 83H4, which has the homology to ctDNA, contained 16 *Bam*HI restriction fragments. With the exception of the restriction fragment that contains the vector pJB8¹¹, all the other 15 fragments co-migrated with mitochondrial *Bam*HI restriction fragments. 83H4 hybridized to 11 ctDNA restriction fragments, of which 6 co-migrated with 83H4 and mtDNA fragments (Fig. 2*b*, tracks 1, 3, 4). Because of the arrangement of these fragments on the physical map of the ctDNA¹², the position of the ctDNA sequences hybridizing to mtDNA could be unambiguously determined (see Fig. 3). The six *Bam*HI restriction fragments found in mitochondrial and chloroplast DNAs are located entirely within the inverted repeat of the chloroplast genome. Four additional ctDNA fragments were expected to, and did hybridize to 83H4 (Fig. 2*b*, track 1). These are the border fragments of the mtDNA homology in the chloroplast genome, and span the junctions of the inverted repeats with both the long and short unique regions of the chloroplast genome.

The published nucleotide sequence of the chloroplast 16S rRNA gene and of the spacer region between the 16S and 23S rRNA^{13,14} genes was used to determine the border of the homologous sequence near the 5' end of the 23S rRNA gene.

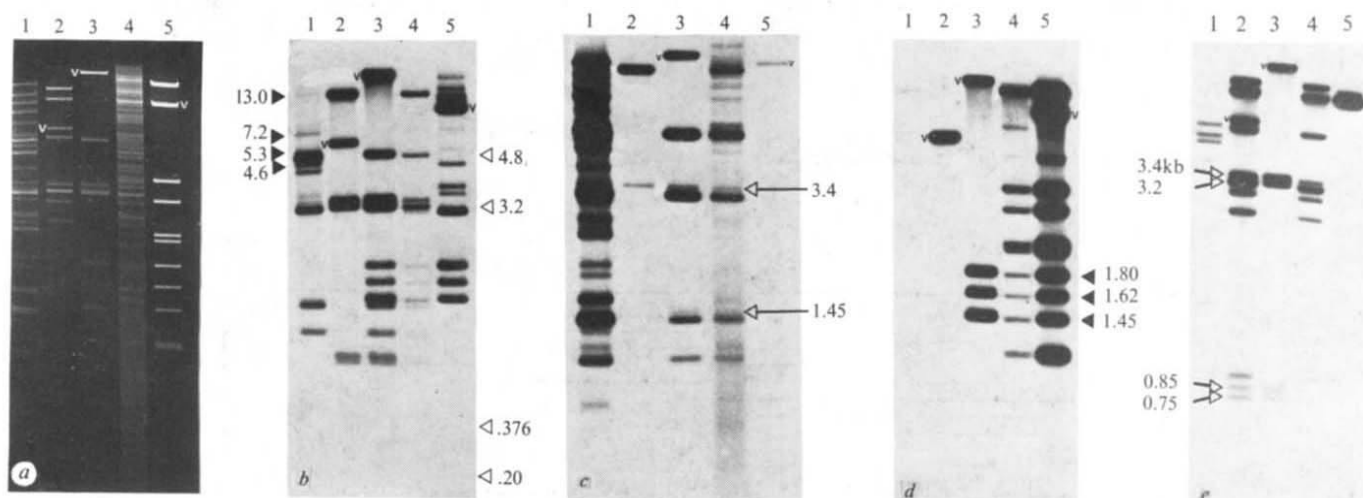


Fig. 2 Homology of mtDNA cosmid clones to mtDNA and ctDNA. *a*, *Bam*HI restriction digests of (1) WF9-N maize ctDNA⁴², (2) 2c87, (3) 83H4, (4) WF9-N maize mtDNA and (5) 91D10 were electrophoresed in 1% agarose gels. The fragments were transferred to nitrocellulose (S&S, BA85) and probed with nick translated 83H4 (*b*), ctDNA (*c*), 91D10 (*d*) and 2c87 (*e*). The sizes of restriction fragments are given in kilobases. Cosmid restriction bands containing the vector sequence are indicated (v). The restriction fragments common to the mitochondrial and chloroplast genomes are indicated (>) in *b*, as are the restriction fragments of the chloroplast genome that border the region of mtDNA homology (>). The mtDNA restriction fragments that border the region of homology with the chloroplast genome are shown (>) in *c*. The restriction fragments shared between 83H4 and 91D10 are shown in *d* (>); those shared by 83H4 and 2c87 are shown in *e* (>). The hybridization to mtDNA is relatively weak because the cosmids are present in approximately a 10-fold molar excess.

Both the mitochondrial and chloroplast genomes have the 1.12-kb *Bam*HI fragment (see Fig. 3a, b). However, the overlapping ctDNA 0.592-kb *Sst*I fragment is absent in the mtDNA and equivalent cosmid sequences. The border of homology, therefore, must be between +1,682 nucleotides and +2,005 nucleotides from the 3' end of the 16S rRNA gene^{13,14}. To the 5' side of the 16S rRNA gene, the 3.4-kb *Bam*HI fragment of 83H4, 2c87 and mtDNA defines the border, as it hybridizes to ctDNA, but is not a ctDNA restriction fragment (Fig. 2c, tracks 2, 3, 4). Fine mapping of this restriction fragment has shown that only 0.6 kb adjacent to the *Bam*HI site shared by the 4.8- and 3.4-kb *Bam*HI fragments hybridizes to ctDNA. Therefore the minimum extent of homology is 12.1 kb.

The cosmid 2c87 hybridizes weakly to four ctDNA fragments of 5.3, 4.6, 4.3 and 1.5 kb. The 5.3-kb (*Bam* 6) and 4.6-kb (*Bam* 8) fragments are the ctDNA fragments that span the borders of the inverted repeat with the long unique region of the chloroplast genome. The remaining two fragments of 4.3 and 1.5-kb derive from the long unique region of the chloroplast genome. Their exact position and the reason for homology to 2c87 have yet to be determined.

The complete conservation of restriction sites for five enzymes, and the alignment of the restriction fragments derived from the chloroplast inverted repeat with the mitochondrial restriction fragments, predicted that the mitochondrial genome contained the chloroplast 16S rRNA coding sequence as well as the split gene for the tRNA^{Ile} and the uninterrupted tRNA^{Val} gene¹⁵. Also, the mitochondrial genome may contain only half of the tRNA^{Ala} coding sequence. Although we have not tested directly for the tRNA coding sequences, 5'-labelled chloroplast 16S rRNA hybridized as expected to the 3.2-kb *Bam*HI fragment of chloroplast, 83H4 and mtDNA (Fig. 4). We are unaware of any other coding functions in this region of the ctDNA.

Integrity of the mtDNA clones

The possibility that this ctDNA sequence has been selectively purified and ligated into this unique region of the mitochondrial genome, creating a set of anomalous clones, can be discounted. MtDNA ($\rho = 1.706$) purification includes banding in neutral CsCl, which should effectively separate it from ctDNA ($\rho = 1.698$). The 5.9-, 5.6- and 1.75-kb *Sst*II restriction fragments of 83H4 are easily identified in restriction digests of mtDNA, and are present in the same stoichiometry as other unique mtDNA restriction fragments. The frequency of recovery of clones from this region is not significantly different from that for other regions of the mitochondrial genome. Thirty-one cosmid clones of mtDNA, which constitute a set of overlapping sequences (Fig. 3e), and span this unique region of the mitochondrial genome have been identified from a total of 544 cosmid clones, from two independently generated clone banks.

Checks for deletions, rearrangements or the ligation of non-contiguous genomic or non-mitochondrial DNA segments into the cosmid clones were made by nick-translating cosmid DNA, and hybridizing it to *Bam*HI, *Sst*II and *Sma*I digests of the respective cosmid and mtDNAs. As these enzymes do not cleave the vector, all the restriction fragments except for the restriction fragment containing the vector and its mtDNA arms should co-align with mitochondrial genomic restriction fragments. If the cosmids, for example 83H4, spanning this region are chimaeras of mitochondrial and chloroplast DNA, generated during the cloning process, ligation of DNA segments which are normally non-contiguous would considerably alter the restriction profile of the cosmid, when compared with the mitochondrial genomic restriction profile. Such discontinuities have not been observed (see for example Fig. 2b, tracks 3, 4; Fig. 2d, tracks 4, 5 and Fig. 2e, tracks 2, 4).

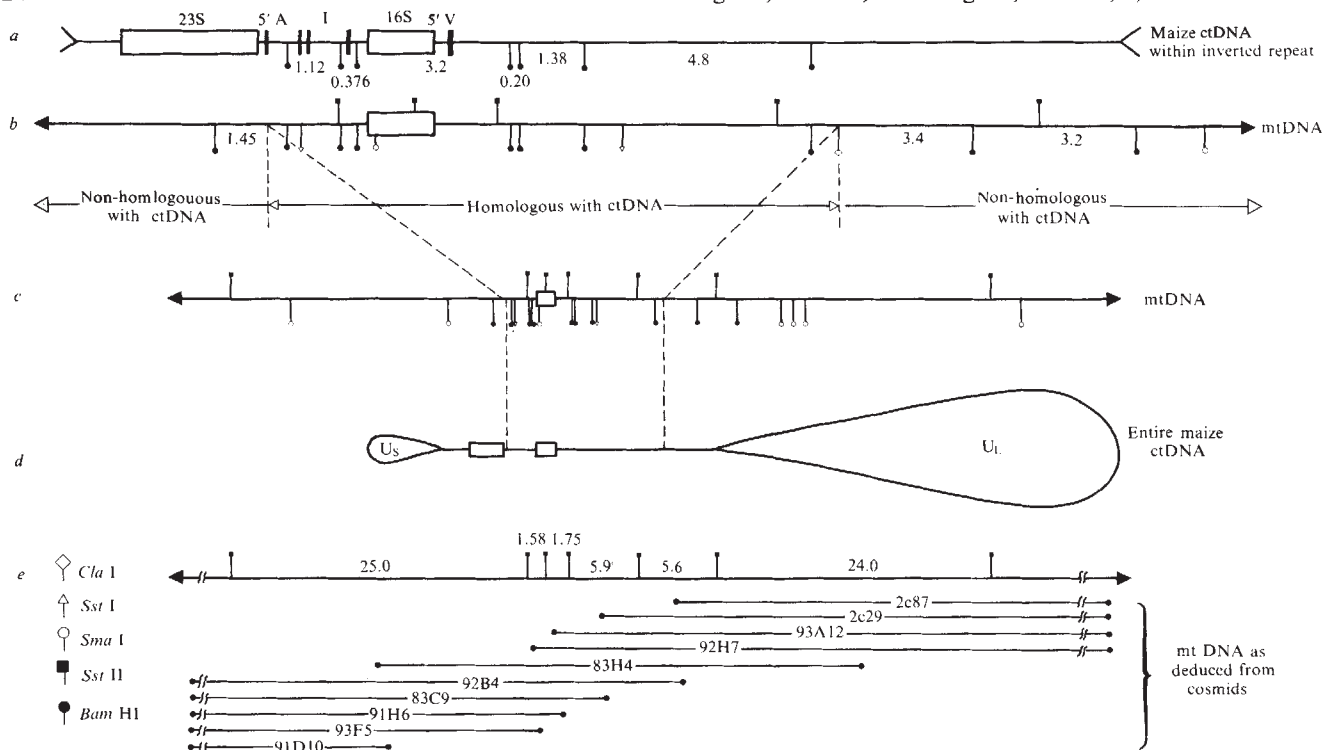


Fig. 3 Restriction endonuclease maps of the inverted repeat regions of maize ctDNA and the homologous region of the maize mtDNA. The physical map of the inverted repeat region of maize ctDNA (a) has been aligned with the homologous region of mtDNA (b); the latter map also includes the flanking, non-homologous regions. The region within the dotted lines defines common restriction fragments between the genomes and the approximate extent of the homologous sequence. The ctDNA sequence contains the genes for 16S rRNA, tRNA^{Val} (V), tRNA^{Ile} (I) and part of the tRNA^{Ala} (A) species in the homologous region¹³⁻¹⁵. A more extensive map of the mtDNA, extending more than 30 kb in each direction away from the homologous region is presented in (c). The map was constructed using standard techniques of single and double digests of cosmids and restriction fragments isolated from agarose gels⁴³. The entire chloroplast genome (d) is shown for size comparison. The loops represent the short unique (U_S) and long unique (U_L) regions, with the 22 kb inverted repeat between them. In (e), representative cosmid clones of mtDNA are shown relative to the *Sst*II restriction map. In total, 31 cosmid clones of this region have been recovered. The positions of the cosmids relative to the *Bam*HI fragments have not yet been determined for each representative clone.

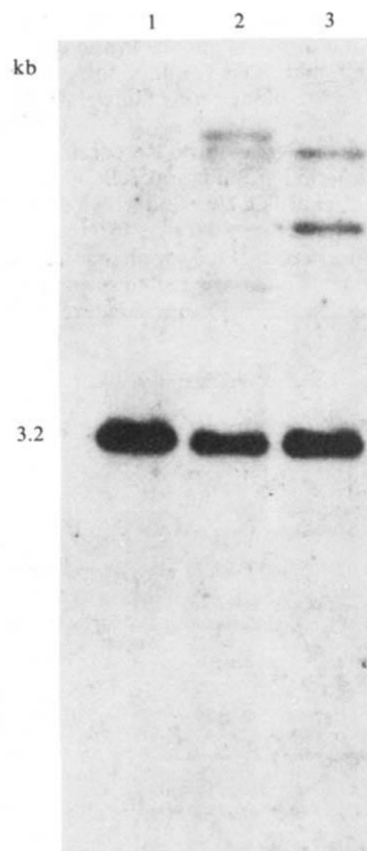


Fig. 4 Chloroplast 16S rRNA gene sequence is present in 83H4 and mtDNA. Equimolar quantities of (1) maize ctDNA, (2) maize mtDNA and (3) 83H4 were digested with *Bam*HI and transferred to nitrocellulose. Hybridization was to 5'-labelled chloroplast 16S rRNA (kindly supplied by Dr T. Dyer) for 16 h at 42 °C in 50% formamide, 0.75 M NaCl, 25 mM PIPES pH 6.8, 5 mM EDTA, 0.2% polyvinyl pyrrolidone, 0.2% Ficoll, 0.2% gelatin and 50 µg ml⁻¹ sonicated, denatured salmon sperm DNA. The filter was washed twice in 2×SSC at 42 °C, and then incubated for 1 h at 20 °C with 10 µg ml⁻¹ RNase A before autoradiography.

Sequence alteration in mtDNA of male-sterile lines of maize

There are differences in the fragments produced by restriction enzyme digestions of mtDNA from three male-sterile (cms-C, cms-S and cms-T) and male-fertile (N) lines of maize^{16,17}. Probing of *Bam*HI digests of the normal and male-sterile lines with 83H4 (Fig. 5a), showed that in cms-T there is a 100-base pair (bp) deletion in the 4.8-kb *Bam*HI fragment, and that there are alterations in the 4.8-, 1.38- and 1.12-kb *Bam*HI fragments in cms-S and cms-C. In addition, the 3.2-kb *Bam*HI fragment is missing in cms-C, indicating that the chloroplast 16S rRNA coding sequence is not present in cms-C mtDNA (Fig. 5b).

No variation has ever been observed in ctDNA restriction profiles from the fertile or male-sterile cytoplasms, with the exception of a single *Hind*III fragment in cms-S ctDNA¹⁶.

Homology of maize mtDNA to other chloroplast genomes

Hybridization of the cosmid clone, 83H4, to restriction digests of wheat, mung bean and *Chlamydomonas* ctDNAs revealed homology to all three. In all three species the homologous region was contained in the inverted repeats, the amount and extent of homology being greatest in wheat and least in *Chlamydomonas* (data not shown). Although homology between three similarly located sequences in the chloroplast genomes of unrelated species is not unexpected, their homology to a maize mtDNA sequence is unexpected, and raises the question as to whether an identical sequence will be found in the corresponding mitochondrial genomes.

Discussion

The presence of a 12-kb, homologous DNA sequence in the mitochondrial and chloroplast DNAs of male-fertile maize is remarkable and unexpected, and has new implications for organellar evolutionary studies. The origins of the mitochondrion and chloroplast are still being debated, although the 'endosymbiont hypothesis' is gaining acceptance, especially for

the chloroplast¹⁸. The high degree of homology between the mitochondrial and chloroplast sequence studied here suggests that whatever the origins of the organelle genomes, recombination or transposition of DNA has occurred between them in the eukaryotic cell and the resulting genetic variant has become fixed in the organelle population. It is possible that a recombination or sequence correcting mechanism operates either in every generation or only rarely during evolution, but sufficiently often to explain the high levels of homology. If there were recombination, fusion between the chloroplast and mitochondrion would have to take place. In the alga *Chlamydomonas*, fusion of chloroplasts¹⁹, and recombination between chloroplast genetic markers^{19,20} has been extensively studied. Similarly, recombination in mitochondrial systems, for example *Aspergillus nidulans*, yeasts and possibly *Paramecium* and tobacco hybrids¹⁹⁻²⁵, has been documented. Although, to our knowledge, mitochondrion-chloroplast fusion has not been directly observed, thin cell sections have revealed outer membrane continuities, as well as more complex connections involving the endoplasmic reticulum, between the two organelles in *Pteris vittata* and *Lycopersicon esculentum*²⁶. Additionally, an interdependence of the two organelles on a polypeptide level has been suggested in certain species²⁷.

If the sequence is maintained by active inter-organellar transport of DNA, then a vector capable of performing this function must exist. Two mitochondrially located linear transposon-like elements²⁸, which on the basis of genetic evidence may be

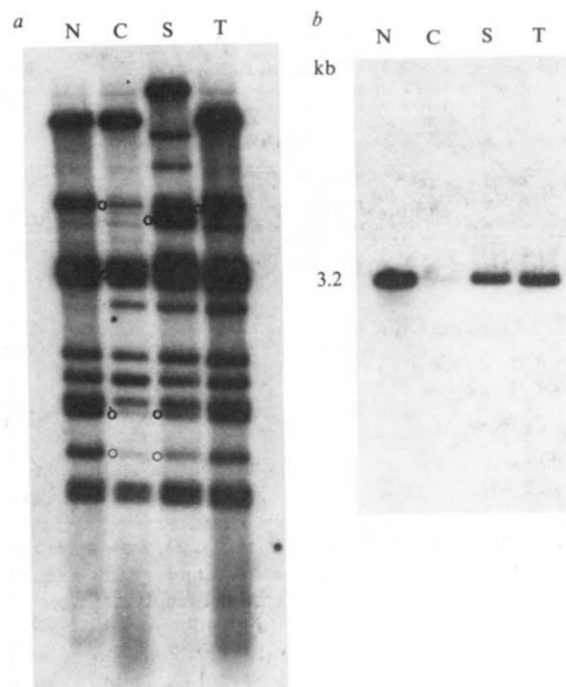


Fig. 5 Analysis of the fertile WF9-N and male-sterile cytoplasmic genomes (cms-S, cms-T and cms-C) in the region of homology. DNAs from mitochondria were extracted, digested with *Bam*HI and electrophoresed in a 0.75% agarose gel. The fragments were transferred to nitrocellulose and hybridized with the ³²P-labelled cosmid 83H4 and autoradiographed (a). The alterations in the male-sterile cytoplasms (0) indicate that part of the chloroplast-mitochondrial homologous sequence has been deleted. To determine whether the 3.2-kb *Bam*HI fragment containing the chloroplast 16S rRNA gene (Fig. 3) was conserved in the male sterile mtDNAs, the 3.2-kb *Bam*HI fragment of 83H4 was cloned into pBR322 and transformed into *Escherichia coli* ED8767⁴⁴. The resulting recombinant plasmid, pZ20, hybridizes to a 3.2-kb *Bam*HI fragment in N, S and T mtDNAs (b). However, it does not hybridize to cms-C mtDNA, although a little residual hybridization, possibly due to the presence of some contaminating ctDNA, is visible. This contamination (in a and b) are for the residual hybridizations in the cms-C, cms-S and cms-T tracks which are equivalent to the fragments in N.

capable of transposition and integration into nuclear loci²⁹, are found in all *cms-S* cytoplasm³⁰. Small circular DNAs are also seen in undigested maize mtDNA preparations^{31,32}. If a vector capable of transporting DNA between organelles were identified, it could prove invaluable for introducing genetic material simultaneously into chloroplasts and mitochondria of the same species.

The alterations in the 12-kb sequence of the male-sterile cytoplasm, reducing the homology between the chloroplast and mitochondrial DNA sequence in these plants, raises the possibility that the mitochondrial sequence is essential for normal plant development. Alternatively, the lack of homology may be because the sequence correction or homogenizing

mechanisms operate only infrequently in evolution or only in fertile plants. The conservation of this sequence in the chloroplast genomes of both normal and male-sterile plants reflects the functional necessity of this sequence for chloroplast, and therefore plant, survival.

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LETTERS TO NATURE

A possible CH subdwarf

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CH giants, with M_V around -1 to 0.5 (refs 1, 2), are stars which have the following well-known properties: (1) weak lines of iron-group elements², (2) rich in carbon, exceptionally strong features of CH, moderately strong (depending on the surface temperature) C_2 and CN (refs 1, 2), (3) relatively strong lines of the s-process elements (especially Sr and Ba), and (4) halo-population kinematics^{3,4}. CH giants are also found in globular clusters: ω Cen, M22 and M55 (refs 5, 6). Later, Bond⁷ discovered a group of field stars, called CH subgiants, with M_V from 2 to 4 (ref. 8). The four properties listed above are shared by the CH subgiants. We discuss here a possible CH subdwarf. First, we establish its subdwarf nature through photometry and proper motion, then we discuss spectroscopic data. Finally, we review how CH giants, CH subgiants and the CH subdwarf constitute a sequence of increasingly challenging cases for the stellar evolution theory.

The star under investigation is designated 2C24 of SA 51. (The photoelectric calibrated photographic photometry⁹ was supplied by Dr I. R. King.) We summarize the relevant data here: $V = 18.79$, $B - V = 0.67$. The absolute proper motion of this star (with respect to galaxies) is $\mu_\alpha = 0.001 \pm 0.003$ arc s yr⁻¹ and $\mu_\delta = -0.011 \pm 0.002$ arc s yr⁻¹ (ref. 10). The errors given

here are those of the relative proper motion. There is an additional uncertainty of ~ 0.002 arc s yr⁻¹ due to the correction from relative proper motion to absolute proper motion. The proper motion was derived from several Lick 3-m reflector prime-focus plates (taken with the Ross corrector B) with 7.5 yr of baseline. Plates taken now would almost triple the astrometric baseline.

The interstellar extinction in SA 51 is small and is adopted to be $A_V = 0.2$ mag (ref. 10). We can now compute the photometric parallax by assuming that it is alternatively a white dwarf, main-sequence star, subgiant or giant. The result is summarized in Table 1. From the photometric parallax and proper motion, we can compute its tangential speed, and the result is also given in Table 1. Based on tangential speeds, it is extremely unlikely that the star is either a giant or a subgiant. Although the possibility that the star is a white dwarf cannot be ruled out, based on the reduced-proper-motion diagram, it is far more likely that the star is a subdwarf¹⁰. The subdwarf nature can be further strengthened by projecting the proper motion of this star in galactic coordinates. The result is $\mu_l = 0.011$ arc s yr⁻¹ and $\mu_b = -0.003$ arc s yr⁻¹. Its proper motion is predominantly in the direction of increasing galactic longitude. In the anticentre direction of SA 51 ($l = 189^\circ$, $b = 21^\circ$), such a positive μ_l implies that the star is trailing behind the circular motion of the Sun with a large tangential speed (~ 200 km s⁻¹), that is, the galactic orbit of the star has very little angular momentum, another characteristic of the halo population.

The claim that 2C24 is a subdwarf rests almost completely on its measured proper motion. According to Table 1, if the star were actually a subgiant, our absolute proper motion must be in error by 4σ of the relative proper motion. Such an error seems remote for a star. Although we have found in one case a 7σ 'motion' for a QSO in SA 57¹¹, it is presumably due to the following reasons: (1) possible variability of QSO's brightness, (2) dissimilar spectra of QSO and (3) heterogeneity of the

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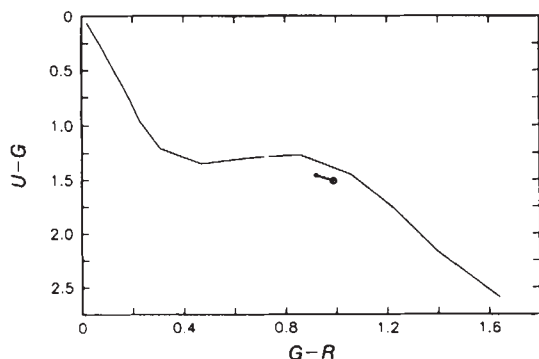


Fig. 1 A $(U-G, G-R)$ two-colour diagram. The continuous line represents the disk population mean main sequence³³. The circle is the location of the star 2C24 which is below the mean main-sequence line. The de-reddening vector is shown below the star.

plates (some plates were taken at large hour angles, and because QSO spectra can differ from each other widely, differential refraction could be very different). In any case, these factors are insignificant for stars.

SA 51 2C24 is the same star as Becker no. 558 (ref. 12). Becker and Fenkart¹² have also published a finding chart for the star. The RGU data for 2C24 are $G = 19.34$, $G-R = 0.99$ and $U-G = 1.51$ (ref. 12). Our motivation for observing this star spectroscopically came from Fig. 1. We have already established 2C24 as a subdwarf and yet its position on the two-colour diagram of the RGU system¹³ mimics a disk population 'metal-rich' star by having a negative UV excess in the RGU system. (However, the random error in $U-G$ for a star of this faintness could be as large as 0.1 mag.)

It would be interesting to investigate more closely the 'halo-type' stars with negative $\delta(U-G)$ among the extensive RGU data. 2C24 falls into this category. They were designated as halo type by Becker¹³ essentially on account of their distances above the galactic plane, despite the fact that they have negative $\delta(U-G)$. (However, some of these stars have negative $\delta(U-G)$ because of photometric errors.)

During an observing run in February 1980, 2C24 was observed along with other calibration objects¹¹ during the first half of the night. We used the Kitt Peak 4-m Mayall telescope with IIDS 'gold' spectrograph. 2C24 and calibration objects were observed with 3.2 arc s round apertures and grating 26 in first order, giving a range $\lambda\lambda$ 3,650–5,400 at about 10 Å resolution.

The spectrum, with a 33.3-min integration time, is displayed in Fig. 2. It appears to have a strong G band of CH and broad absorption features (due to the absorption of P , R branches of the CH molecule) flanking both sides of the G band. This absorption is a signature of the CH star. The absorption trough strongly affects the B passband such that CH stars have positive $\delta(U-B)$ in the UBV system. The G passband of the RGU system, however, lies towards longer wavelengths (see ref. 14 for RGU response functions), and it is hardly affected by this feature. For this reason, 2C24 has a negative $\delta(U-G)$ in the RGU system. In the presence of $[C/Fe]$ variation, $\delta(U-G)$ is no longer solely dependent on $[Fe/H]$.

The KPNO spectrum for 2C24 is of low signal-to-noise ratio, but perhaps a more serious limitation is that none of the standard stars that were observed¹¹ are as red in $B-V$. Therefore, the star was placed on the McDonald 2.7-m observing program for additional integration, using an IDS detector built by P. Rybski. We used the 'blue chain' for a 45-min integration in photometric conditions on 5 December 1980. The observational specifications (aperture size, dispersion, resolution, spectral range) were all closely similar to those of the KPNO data. We also observed SA 51 88S and SA 51 107S which bracket 2C24 in $B-V$. The standard stars were observed both before and after 2C24 as a test for spectrophotometric drifts (none were apparent). The results are shown in Fig. 3. The spectrum

of 2C24 seems to show a strong Ba II line at $\lambda 4,554$, and a G band that is quite unlike that seen in the standard star 88S. Indeed, the shape of the continuum over the whole interval $\lambda\lambda$ 4,200–4,400 appears depressed. The Ba II line appears on one of the two KPNO scans co-added in Fig. 2; the other scan could have been affected by an ion glitch.

The spectroscopic evidence that 2C24 is a subdwarf CH star is not strong. Figure 2, for instance, resembles the comparison star spectrum of Fig. 3b, and the KPNO and McDonald spectra are dissimilar. We favour the data presented in Fig. 3 because of the spectrophotometric precautions mentioned above. Clearly, more data are needed to confirm our claimed features, but that would require a commitment of large telescope time. An easier check involves good broadband photoelectric colour measurements, as suggested by Mannery and Wallerstein¹⁵.

In view of the strength of the apparent Ba II $\lambda 4,554$ line in the McDonald spectrum, one might wonder whether 2C24 should be classified as a Ba II star. Indeed, the Ba II stars also have enhanced CH bands¹⁶. We have adopted the term CH star for 2C24 simply because it has halo-population kinematics. Another deciding factor would be the abundance of iron-group elements. However, the low resolution and poor signal-to-noise ratio of our spectra do not support a detailed chemical analysis.

Neither the enhanced carbon (attributed to the triple- α process) nor the absolute magnitudes of CH giants pose much difficulty to stellar models, although the mixing needed to dredge the carbon to the stellar surface can be a problem. Production and mixing of s-process elements present further difficulties in CH giants.

In the case of CH subgiants and the proposed CH subdwarf, if we assume that CH stars are single low-mass stars ($\sim 1 M_{\odot}$) with carbon and s-process elements produced from the interior, we would be confronted by more severe problems. First, the triple- α process requires a temperature of $\sim 10^8$ K (ref. 17), but the interiors of such stars are not at such a high temperature. One way to reconcile this dilemma is to postulate that they have already gone through the stage of helium flash to produce carbon. Second, the mixing process is still needed to explain the enhanced carbon and s-process elements in the stellar atmosphere. In the case of the CH subdwarf, enough hydrogen has to be mixed into the interior to rekindle the core hydrogen burning (and thus re-enter a main-sequence phase). Third, the intrinsic luminosity of the model stars must be low enough to be compatible with those of the CH subgiants and the CH subdwarf.

Two mixing mechanisms often invoked are: the helium core flash at the tip of the giant branch and helium shell flashes on the asymptotic branch. Stellar models on the asymptotic branch do not predict the needed mixing of carbon from the intershell zone^{18–20}; s-process elements are not formed in these models. Furthermore, the intrinsic luminosity of these models turns out to be too bright for CH subgiants ($M_V \leq -1.3$)²¹, let alone a subdwarf. Conventional models of stellar evolution do not predict mixing at the core flash either²² unless the core is artificially allowed to grow very large before the flash^{23,24}.

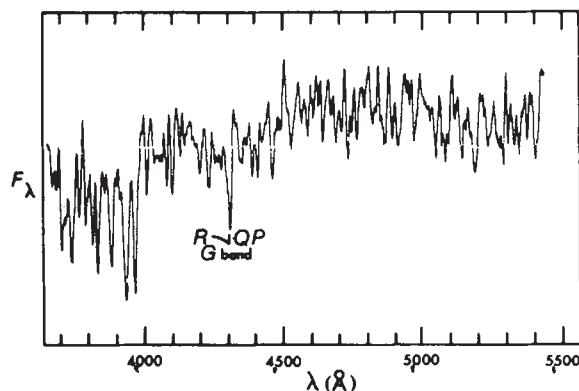


Fig. 2 IIDS spectrum of 2C24 obtained at KPNO.

Table 1 Distances and tangential speeds with various assumptions

Assumed luminosity class	M_V	r	z	T
Disk population white dwarf	14.4	69 pc	25 pc	3.6
Halo population subdwarf	5.7	3.8 kpc	1.4 kpc	2.0×10^2
Halo population subgiant	2.5	17 kpc	5.9 kpc	8.6×10^2
Halo population giant	-0.5	66 kpc	24 kpc	3.5×10^3

r , Photometric distance; z , $r \times \sin(21^\circ)$, that is, the distance above the galactic plane; T , tangential speed in km s^{-1} .

Smith and Demarque²¹ have assumed the existence of a mixing event in spite of these problems, and they also take into account the additional effects of (1) losing mass, (2) mixing fully^{25,26} or partially²⁷, (3) mixing centrally or noncentrally (due to neutrino processes^{23,28}). After detailed calculations, they concluded that only models that have not lost mass, either before the mixing or after, are faint and cool enough to represent CH subgiants, and only fully mixed models with mass loss after the mixing process could be as faint as 2C24. It is by no means certain that s-process elements can be formed in this picture. Thus, with the *ad hoc* assumptions of mixing and mass loss, the problem of absolute luminosity can be explained, but we are left with the problems of the origin of mixing and the production of s-process elements.

The possibility that CH stars are in binary systems seems very attractive in view of the difficulties we encountered above and in view of the fact that Ba II stars, the disk-population counterpart of the CH stars, are in binaries²⁸. Further work to establish the binary nature of CH stars is of considerable interest²⁹.

The existence of a carbon enhancement in low-luminosity, high-velocity stars is not unprecedented. Dahn *et al.*³⁰ discussed the case of G77-61, a dwarf with very strong bands of C_2 as well as CH. The star GH 7-21³¹ has very similar colours to 2C24, and GD 439³² resembles 2C24 in having strong CH but no Swan bands. The claimed detection of Ba II in a subdwarf is unique, however, and should be confirmed.

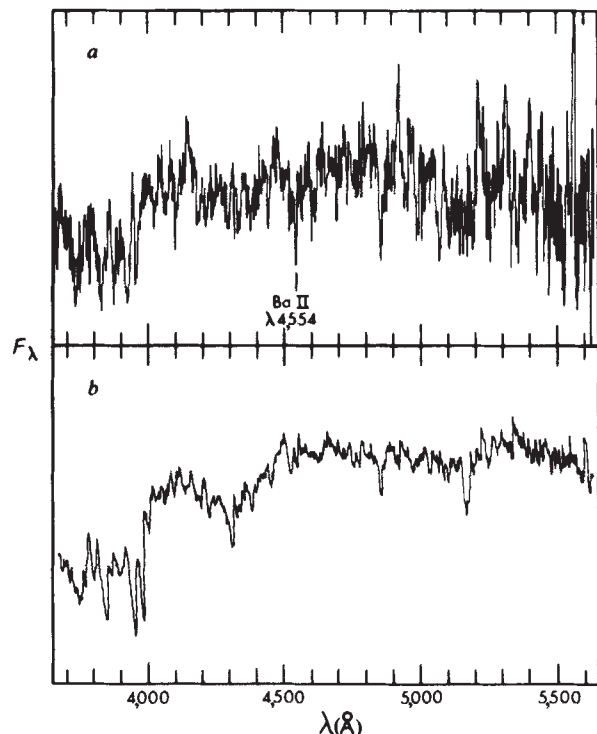


Fig. 3 McDonald spectrum of 2C24 (a) and comparison star 88S (b). The comparison star 88S is a disk population main-sequence star based on its reduced proper motion¹⁰

We thank the staff of the Kitt Peak National Observatory for assistance of reducing IDS data on the IPPS. The McDonald spectra (Fig. 3) were produced by Mr A. Jankevics. We also thank Drs Dennis Butler, Pierre Demarque, Jim Liebert, Bob McClure and Jas Smith for illuminating discussions. L.-T.G.C. was partially supported by NSF AST 76-81339 to Dr William F. van Altena. Publication costs were in part defrayed by NSF AST 80-12725 to Dr van Altena. L.T.G.C. and R.G.K. are visiting astronomers at the Kitt Peak National Observatory, which is operated by the Association of Universities for Research in Astronomy, Inc., under contract with the NSF.

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Solar core rotation

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In the continuing study of solar oscillation by means of optical resonant scattering¹, two sets of data were obtained between June and August 1981. The first of these was at Haleakala on the island of Maui and consisted of 70 days of data over a period of 83 days, whilst at Izana on the island of Tenerife 85 days were obtained from an observing season of 88 days. At Izana a total of 895.5 h of data gave an average of 10.5 h per day, whilst on Haleakala an average of 10.1 h per day was achieved. As the two sites are ~9.5 h apart partial overlap of data often occurred. These data represent the apparent line of sight velocity between the solar surface and the observer. It is the analysis of such data that has led to the discovery of the 160-min oscillation², the structure and global nature of the 5-min oscillations³ and recently, the rotational splitting⁴ of these latter oscillations gave the first conclusive experimental evidence that the solar core is rotating more rapidly than the observable surface. The new data on solar line of sight velocity measurements reported here, establish the existence of a 13.1 ± 0.2 day (synodic) signal of amplitude 6.5 m s^{-1} . This is variously interpreted in terms of a rapidly rotating solar core.

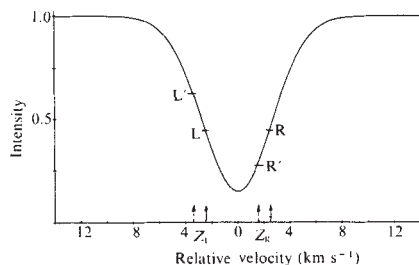


Fig. 1 A diagrammatic representation of the potassium 769.9-nm Fraunhofer absorption line illustrating the mean relative position of the laboratory Zeeman components at two different epochs.

The availability of the present long data stretches makes it possible to search for longer periods, several days, which may be associated with a rapidly rotating solar core. Indeed, Dicke⁵, analysing a 97-day observational period containing 47 days of data on determinations of the apparent solar oblateness, found a 12.64-day (synodic) periodic signal which was attributed to a rigidly rotating solar distortion.

The Doppler shift line of sight velocity measurements depend essentially on the comparison of a given Fraunhofer absorption line, potassium 769.9 nm in the present instance, with a laboratory reference standard. A stable vapour of potassium is used and when placed in a magnetic field the two Zeeman components (Z_L , Z_R) of the appropriate line sample the solar line as shown in Fig. 1. By alternately measuring the intensities (I) at L and R the relative positions of the solar and laboratory lines may be readily found by considering the ratio

$$R = \frac{I_L - I_R}{I_L + I_R} \quad (1)$$

Clearly if there is no relative displacement of the solar and laboratory lines (before Zeeman splitting), this ratio would be zero. Any finite value is attributable to Doppler velocity shifts, gravitational shifts and instrumental effects. Hence the observed ratio, converted to the equivalent Doppler velocity may be

expressed as,

$$V = V_{\text{orb}} + V_{\text{spin}} + V_G + V_I(t) + V(t) \quad (2)$$

where V_{orb} is the line of sight component due to the Earth's orbital motion, V_{spin} that due to the Earth's rotation, V_G shifts due to the gravitational potential, $V_I(t)$ possible time-dependent instrumental effects and $V(t)$ the signal due to solar surface oscillations. The first two terms are accurately known and provide a means of absolute calibration of the apparatus. The Earth's rotation gives a $\sim 400 \text{ m s}^{-1}$ sinusoidally varying signal during any one day, whilst V_{orb} changes by $\sim 700 \text{ m s}^{-1}$ over the observing season. This has the effect of moving the centroid of the solar line relative to the laboratory line so that the Zeeman components, (dotted) sample different regions of the Fraunhofer absorption line as illustrated by the points L', R' in Fig. 1. Indeed it is only during September/October that the effects of V_{orb} and the gravitational shift V_G combine to produce a symmetrical scan of the line by the Earth's rotation. As the solar line is approximately gaussian in profile, the slopes at points such as L', R', are noticeably different and result in the purely instrumental velocity component $V_I(t)$ which varies with the observing season.

Alternate measurements of the resonantly scattered light intensity (I_L , I_R) were recorded at 1-s intervals and the mean ratio as defined by equation (1) over a 42-s interval was used as the primary data for further reduction. This gave a series of typically 900 points per day which were fitted by an expression of the form

$$A + Bt + C \sin \omega(t - t_e) \quad (3)$$

where B represents the known change in orbital velocity during the day, typically 0.3 m s^{-1} per h, C the Earth's rotational velocity ($\sim 400 \text{ m s}^{-1}$), ω is the angular frequency corresponding to the Earth's 24-h rotation period and t_e is the time of ephemeris transit. Hence by finding A and C from a fit to the data and evaluating the absolute sensitivity of the apparatus (C), the mean apparent line of sight velocity for the day is given by

$$A = \bar{V}_{\text{orb}} + \bar{V}_G + \bar{V}_I(t) + \bar{V}(t) \quad (4)$$

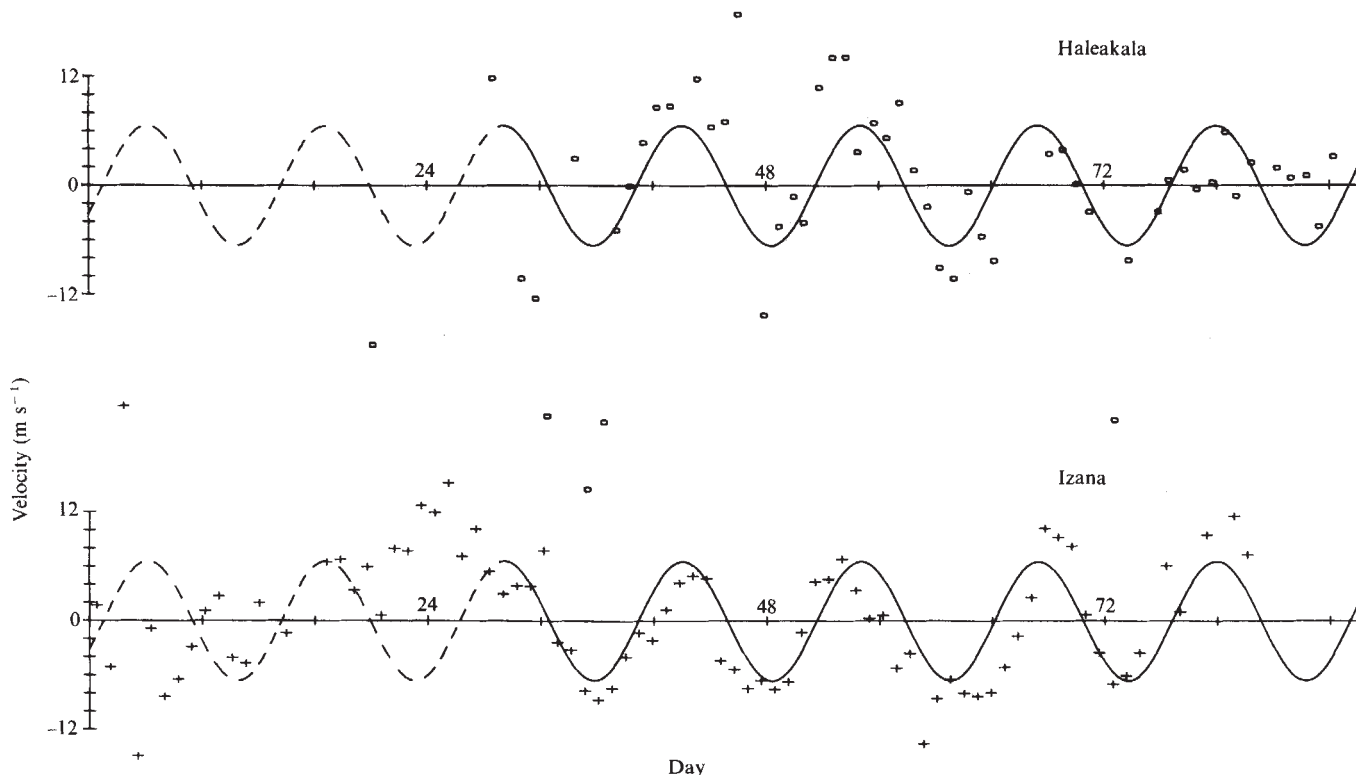


Fig. 2 A comparison of the 1981 data obtained at Haleakala and Izana, after correcting for line asymmetry, for the period 29 May to 22 August (starting JD 244, 4752). The superimposed curve is that obtained from a best fit to the Izana data over the period 28 June to 22 August.

The value of $\bar{V}_{\text{orb}}$, reflecting not only the orbital motion of the Earth about the Sun but also the influences of the planets and the Moon, may be readily found from the Astronomical Almanac. The trend due to the line asymmetry over the operating points, represented by $\bar{V}_1(t)$, may be fitted by an equation of the form

$$Y = \gamma + \alpha e^{-\beta t} \quad (5)$$

and after removal yields the data points shown in Fig. 2.

The data obtained at Izana clearly show the existence of an oscillatory signal although the earlier values are subject to uncertainties associated with short data stretches and poorer weather conditions. These latter effects may obscure the oscillatory signal or it may indeed not be detectable and only manifests itself at a later date.

The data taken at Haleakala during the same observing period were treated similarly and are shown for comparison in Fig. 2. Unfortunately observing conditions on this site were not as favourable and a consequent increase in the amount of random scatter is evident. Nevertheless, the existence of the oscillation is evident and is in phase with that observed at Izana. Bearing in mind that the two sites are situated at approximately diametrically opposite points of the Earth, the correspondence of the two sets of data clearly demonstrates that the signal is of solar origin.

To determine the frequency spectrum of the observed signal, those data obtained at Izana between 28 June and 22 August are subjected to an iterative sine wave fitting procedure. The spectrum of amplitude squared against frequency is plotted in Fig. 3. The peak at $0.88 \mu\text{Hz}$ has a width which is consistent with the intrinsic resolution of the data string ($1/T \sim 0.2 \mu\text{Hz}$) and corresponds to a synodic period of 13.15 days. This best fit signal is superimposed on both sets of data shown in Fig. 2, the solid curve indicating the region over which the fit has been made.

Observational data from previous years confirm the presence of the ~ 13 -day signal but both quality and length of data are less than that obtained in 1981. Table 1 summarizes the available data and the signals found.

The 1978 data consisted of only 12 contiguous days and consequently no attempt was made to determine a period from these data, however, the relative phase, as defined by a minimum in the signal, could be found. The period found for the 1980 data reflect the uncertainty introduced by considering shorter data strings. Considering all the available data to be coherent, a signal of period 13.05 days may be found which is consistent with the phase and frequency determinations found for individual years. Thus the best estimate of the period, based on all available data, is 13.1 ± 0.2 days (synodic). Considering the full span of data alternative solutions of 12.59 and 13.54 days may be found but are deemed to be less likely.

The velocity amplitude of 6.6 m s^{-1} found for the $0.88\text{-}\mu\text{Hz}$ peak implies a mean surface displacement of $\sim 1 \times 10^6 \text{ m}$ or a fractional change in solar radius of 1×10^{-3} . This seems inordinately large and would seem to suggest that some mechanism

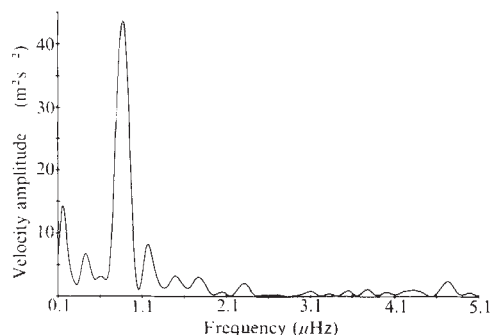


Fig. 3 An amplitude squared spectrum obtained from an iterative sine wave fit to the 1981 Izana data for the period 28 June to 22 August.

Table 1 Dates of observations with the number of useful days used in the analysis to find the periods and amplitudes shown

Year	Observation period	Days of data	Period (synodic) (days)	Amplitude (m s^{-1})	Phase (minimum)
1977	12 July–24 August	35	13.08	6.5	13 August
1978	31 July–11 August	12	—	—	3 August
1980	21 July–17 August	28	16.53	5.2	2 August
1981					
Izana	28 June–22 August	55	13.15	6.6	13 August
Haleakala	28 June–22 August	50	13.38	9.8	13 August

Phase refers to a well defined minimum in the periodic signal.

other than a purely Doppler velocity shift due to surface displacement is responsible for the observed signal.

An inhomogeneous mass distribution in the solar interior rotating with a 13.1-day period, could possibly, due to the combined effects of increased gravitational potential causing a spectral shift in the solar absorption line, together with an actual displacement of the solar surface, be responsible for the observed signal. However, the magnitudes involved make this seem unlikely. It may be further speculated that the inhomogeneous rapidly rotating core contributes to the maintenance of the highly coherent global oscillations of the Sun observed in the 5-min region³.

Possible residual circular polarization sensitivity of the apparatus would, through the Zeeman effect, render the system subject to solar magnetic field changes. However, data recorded with full polarization sensitivity during a maximum in the 13.1 days signal indicate that the integral solar disk magnetic field is equivalent to a velocity of $< 1 \text{ m s}^{-1}$.

It has recently been suggested⁶ that the rotational splitting of non-axisymmetric oscillations may be responsible for periods of ~ 13 days. However, the amplitudes of the individual lines is $\sim 0.1 \text{ m s}^{-1}$ and it seems unlikely that the many discrete frequency lines could produce a coherent signal of $\sim 6 \text{ m s}^{-1}$.

Finally the observed signal may be caused by an outflow of material at a specific region of the solar subsurface, not coincident with the rotation axis, which is rotating with a 13.1-day period. A flow of material along, for instance, an oblique rotating magnetic axis^{5,7} would seem possible. An observational study of the solar surface in two dimensions for large scale velocity fields is already in progress⁸ and should help elucidate the origin of the 13.1-day signal.

A comparison of the data must be made with those of Dicke⁵, who when measuring the solar oblateness found, from purely physical displacements of the solar limb, that a signal of $\sim 9 \text{ m arcs}$ at a period of 12.64 ± 0.12 days (synodic) existed. The match of periods is satisfactory but the measured oblateness signal corresponds to a fractional change in solar radius of 1×10^{-5} . Thus if the two observations correspond to the same physical process the interpretation in terms of a distorted gravitational potential⁵ implies that the line shift is the major contributor.

More recently, a study of daily sunspot numbers over the past 122 yr (ref. 9), has shown a 12.0715 ± 0.002 -day periodicity. If all the observations have an origin in the same physical phenomenon, this could be associated with a solar core rotating at approximately twice the observed surface rotation rate.

The interpretation in terms of a core rotating with a synodic period of ~ 13 days is also consistent with the recent results obtained on rotational splitting of the 5-min solar oscillations⁴ and if both results are considered would indicate not only a rapidly rotating solar interior but would establish the radius of the core to be nearly that of the Sun itself.

The picture that emerges from the present observed 13.1-day periodicity and the rotational splitting of the 5 min oscillations, is a Sun with the major part of the solar interior rotating at about twice the observed surface rotation rate. Mass flow along an oblique rapidly rotating axis may explain the observations and is the subject of further investigation.

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Use of γ -zirconium phosphate for Cs removal from radioactive waste

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Radiation stability of synthetic organic ion-exchange resins is not sufficient for the processing of very highly radioactive solutions because these solutions cause significant changes in the capacity and selectivity by hydrolysis of functional groups, chain scission and changes in degree of crosslinking^{1,2}. Among the inorganic ion exchangers, zeolites and clay minerals have been used in decontaminating radioactive waste solutions^{3–6} because of their high ion-exchange capacity, selectivity and presumably their radiation stability⁷; however, their use is limited because of their instability in mildly acid solutions¹. Another class of materials—insoluble acid salts formed by polybasic acids and certain hydrolysable polyvalent cations—also possess cation exchange properties. These salts include phosphates, arsenates, tungstates and molybdates of zirconium, thorium, titanium and other metals⁸. Among these insoluble acid salts, zirconium phosphate gels have been investigated⁹ very extensively. Crystalline phases of zirconium phosphate were first prepared in 1964 and 1968 from gels on refluxing in phosphoric acid by Clearfield and co-workers^{9,10}. We report here the discovery of crystalline γ -zirconium phosphate as a highly selective caesium ion sieve which can be used to separate ¹³⁷Cs from accident waste water or from circulating water in nuclear reactors.

The crystalline phases of zirconium phosphate have layer structures similar to 2:1 clay minerals^{11–13}. The crystalline phase with a composition $\text{Zr}(\text{HPO}_4)_2 \cdot \text{H}_2\text{O}$ is called α -zirconium phosphate (α -ZrP) while $\text{Zr}(\text{HPO}_4)_2 \cdot 2\text{H}_2\text{O}$ is called γ -zirconium phosphate (γ -ZrP). (The extensive literature on the ion-exchange properties of α -ZrP is summarized in refs 14–17, but very little work has been reported on γ -ZrP as revealed by a computer search of the literature.)

Self-diffusion coefficients of calcium and sodium ions were measured^{18,19} in the calcium and sodium ion forms respectively of γ -ZrP. Half and fully exchanged alkali and divalent transition metal forms of γ -ZrP were prepared^{20–22}. Half-sodium exchanged γ -ZrP has been used for the separation of strontium from radioactive waste water²³. However, there is little or no work on the selective uptake of Cs from radioactive waste solutions by the γ -ZrP. Caesium is one of the hazardous and major fission products that needs to be separated from circulating water in all nuclear reactors and accident waste water such as at the Three Mile Island reactor. The research reported here shows that γ -ZrP is extremely selective for Cs in such solutions containing H^+ , Na^+ and Ca^{2+} .

Crystalline γ -ZrP was synthesized by Yamanaka²⁴. An adsorption isotherm (Fig. 1) was determined for Cs by equili-

Table 1 Cs sorption from simulated waste solutions

Ion exchangers	Cs sorption K_d^* (ml g ⁻¹) from solutions		
	Three Mile Island waste	0.04 M NaNO ₃	0.01 M CaCl ₂
γ -ZrP	1,800	16,000	27,700
α -ZrP	—	3,400	1,300
Ionsiv, IE95	800	7,100	11,300
Chabazite, Christamas, AZ	1,000	5,700	16,300
Chabazite, Bowie, AZ	1,100	7,800	24,700
Clinoptilolite, CA	1,100	4,400	16,600
Mordenite, NV	1,800	4,300	165,000
Mordenite, AZ	1,800	5,800	96,500
Erionite, NV	1,800	11,200	29,500
Phillipsite, NV	1,800	9,800	34,500
Cooper micaceous vermiculite, SC	150	—	1,400
Williams vermiculite, SC	—	—	1,100
Poole vermiculite, SC	—	—	800

*Defined as the ratio of the amount of Cs sorbed per g of sorbent to the amount of unsorbed Cs per ml of solution.

brating (shaking) 10 mg of γ -ZrP for 110 h dispersed in 25 ml CsCl solutions containing 33–333 μg per ml Cs. Adsorption of Cs by γ -ZrP reached a plateau (Fig. 1) at about 180.7 ± 6.1 mg per g or about 1.36 ± 0.05 mequiv. g⁻¹ which is its total capacity for Cs sorption at the solution pH resulting from $\text{Cs}^+ \rightleftharpoons \text{H}^+$ exchange. However, when γ -ZrP was titrated with CsOH to a pH of 6.56 the capacity obtained is 2.52 mequiv. g⁻¹. This capacity is similar to the capacity obtained by Clearfield and Garces²¹ and supports their results. Thus, the caesium uptake of γ -ZrP is dependent on the pH of the solution. The total capacity for Cs is smaller²¹ than the total exchange capacity (6.24 mequiv. g⁻¹) of this crystalline γ -ZrP. However, this total Cs capacity of γ -ZrP is still much higher than the total exchange capacity of smectite clay minerals and some zeolites²⁵.

Experiments were conducted for $\text{H}^+ \rightleftharpoons \text{Cs}^+$ exchange isotherm (Fig. 2) after 10 days of equilibration with constant amounts of γ -ZrP, water and total cations, but variable proportions of Cs^+ and H^+ ions. The dashed line in exchange isotherm (Fig. 2) indicates equal preference²⁶ for both the cations. If the exchange isotherm falls above this line, Cs is preferred over H^+ and if the isotherm falls below this line, H^+ is preferred over Cs^+ . The $\text{Cs}^+ \rightleftharpoons \text{H}^+$ exchange isotherm (Fig. 2) shows that Cs^+ is extremely selective at low Cs concentrations up to a loading of $\sim 1.49 \pm 0.15$ mequiv. g⁻¹ of γ -ZrP and after that H^+ is preferred over Cs^+ . The lack of selectivity for Cs^+ after a loading of 1.36–1.49 mequiv. g⁻¹ can be attributed to lack of Cs diffusion due to collapse of the basal spacing to ~ 11.86 Å from 12.25 Å as revealed by X-ray diffraction. This decrease

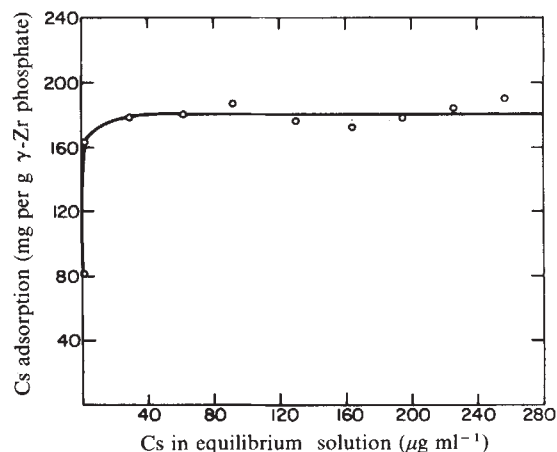


Fig. 1 Adsorption of Cs from solutions by γ -ZrP.

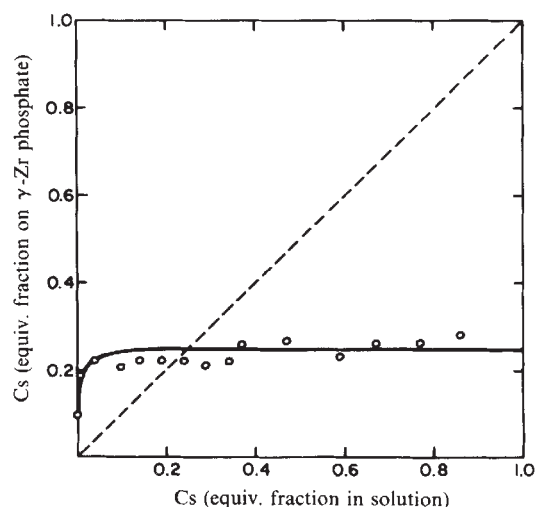


Fig. 2 Adsorption in systems with constant amounts of γ -ZrP water and total ionicity but variable fractions of Cs^+ and H^+ .

in interlayer spacing of γ -ZrP on Cs loading was also observed before by Clearfield and Garces²¹. The Cs loading (1.49 ± 0.15 mequiv. g^{-1}) in the presence of H^+ is the same as the Cs loading (1.36 ± 0.05) in the absence of H^+ within experimental errors and indicates that H^+ gave little or no competition for Cs^+ up to the maximum Cs loading in γ -ZrP.

γ -ZrP, α -ZrP, vermiculites and several zeolites including Ionsiv IE-95, which is presently being used in the submerged Demineralizer System at Three Mile Island (TMI), were treated with a simulated (non-radioactive) waste solution. The simulated liquid waste contained 1,275, 2,935, 1.15 and 0.075 $\mu\text{g ml}^{-1}$ or 0.055, 0.271, 8.7×10^{-6} and 8.6×10^{-7} M of Na, B, Cs and Sr respectively. These concentrations are the midpoints of concentration ranges (S. Pillay personal communication) of varying high specific activity liquid wastes at TMI. 250 mg of each sample was treated with 20 ml of the simulated waste solution for 1/2 h in a batch method and this solid-to-solution ratio represents ~ 200 column volumes. In another series of experiments, 20 mg of various ion exchangers were equilibrated by shaking for 24 h in a 25 ml simulated waste solution of 0.04 M NaNO_3 containing 2×10^{-4} M Cs and 1×10^{-4} M Sr or in 25 ml of 0.01 M CaCl_2 containing 2×10^{-4} M Cs. The Cs sorption by various ion exchangers was determined by separating the solid and solution phases and by analysing the solutions for Cs by atomic absorption spectroscopy. The results are presented as K_d (ml g^{-1}) values in Table 1. These results show that γ -ZrP acts as an extremely selective caesium ion sieve by separating Cs from concentrated solutions of Na^+ and Ca^{2+} . Some of the zeolites treated with TMI waste solution seem to be as good as γ -ZrP for Cs sorption (Table 1). This is because of the high solid-to-solution ratio used which resulted in almost complete Cs uptake. However, γ -ZrP is clearly superior to the zeolites for Cs sorption when low solid to solution ratio was used as with 0.04 M NaNO_3 . Some zeolites are better than γ -ZrP for Cs sorption from 0.01 M CaCl_2 (Table 1).

In most reactors the concentration ranges of ^{137}Cs experienced in circulation water (that is, non-accident conditions) are likely to be very low that is, pg ml^{-1} . To test whether γ -ZrP is selective for low concentrations of ^{137}Cs , another experiment was conducted. 10 mg of γ -ZrP was equilibrated with 20 ml of 0.04 M NaNO_3 containing 50 pg ml^{-1} of ^{137}Cs (Na to Cs equivalent ratio is $\sim 1 \times 10^6$). After equilibration, the solid and solution phases were separated by centrifugation and the amount of Cs adsorbed by γ -ZrP was estimated by determining the ^{137}Cs in the solution using the scintillation method. Results of this experiment indicate that 68% of the added Cs was taken up by γ -ZrP ($\text{Cs } K_d = 4,250 \text{ ml g}^{-1}$) even though there is 100 million times more Na than Cs. Thus, γ -ZrP is highly selective for ^{137}Cs even when it is present at pg ml^{-1} concentration level

and therefore it can be used to clean up ^{137}Cs from circulation water from all nuclear reactors.

γ -ZrP is extremely selective for Cs because of its layer structure²⁴ where the interlayer spacing is about 2.85 Å with a basal spacing of 12.25 Å. Because of this narrow spacing, less hydrated Cs^+ ions are preferred over the more hydrated Na^+ and Ca^{2+} ions. γ -ZrP did not selectively sorb Sr^{2+} from 0.04 M NaNO_3 ($\text{Sr } K_d = 140 \text{ ml g}^{-1}$) because of the steric hindrance. Thus, these results show that Cs^+ can be separated very selectively from Na^+ , Ca^{2+} and H^+ containing solutions such as circulation water in all nuclear reactors or accident waste water. Because γ -ZrP is very acid resistant compared with the zeolites, vermiculites or montmorillonites, this layered ZrP would be very useful for separating Cs from acidic nuclear waste solutions also. In fact, ZrP gel was recently found to be better than clinoptilolite for Cs sorption from acid solutions²⁷. Further work is needed to determine the suitability of this γ -ZrP for column operations.

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Surface studies on a leached sphene glass

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Titanates and titanate- or titanasilicate-based ceramics are being considered as possible nuclear waste hosts in several national waste management programmes^{1–4}, largely because of their excellent resistance to leaching. The leach resistance of titanates has been attributed to the formation of largely insoluble, titanium dioxide rich layers, formed at the surface by selective leaching of univalent and divalent cations⁵. We have examined the leaching of the titanasilicate sphene (CaTiSiO_5) in the form of natural samples, sintered ceramics and glasses of stoichiometric sphene composition. We report ESCA (electron spin for chemical analysis) or XPS (X-ray photoelectron spectroscopy) and SIMS (secondary ion mass spectrometry) studies of leached CaTiSiO_5 glasses, demonstrating the nature of leached surface layers on a titanasilicate. These results also illustrate the parallels between the leaching of sphene glasses in deionized water and the leaching mechanism proposed for titanates⁵.

CaTiSiO_5 glasses were prepared by melting an intimately mixed charge of calcium carbonate, titanium dioxide and silica at 1,450 °C for 1 h, followed by casting 1-cm diameter cylinders in stainless steel moulds. The tendency of the glass to crystallize or phase separate was avoided by keeping the composition within the region where sphene glasses are relatively stable (Fig. 1) and by casting relatively small cylinders (~2 cm length $\times$ 1 cm diameter) which could be cooled rapidly. After annealing at 750 °C the cylinders were cut and polished to give disks 2 mm thick. The bulk composition of the disks was determined by electron microprobe to be close to stoichiometric CaTiSiO_5 (given in brackets) $\text{CaO} = 28.5\%$ (28.6), $\text{TiO}_2 = 40.5\%$ (40.8), $\text{SiO}_2 = 30.0\%$ (30.6).

Samples were leached in deionized water in Teflon bottles at 90 °C. After removal from the leachant, disks were rinsed with deionized water and stored in a desiccator until mounted in the XPS equipment. Spectra were recorded on a MacPherson 36 instrument using an aluminium anode. Element ratios were determined from XPS spectra as reported elsewhere⁶.

SIMS profiles were run on an ARL IMMA instrument using an O_2^+ beam. Sputtering depths were assessed from interference microscope images of the beam impact craters and are probably subject to error limits of $\approx 30\%$. To calculate element ratios, counts for element intensities were normalized to the bulk composition, assumed to be represented by the flat region in the sputtering profile of an unleached sample (see Fig. 3).

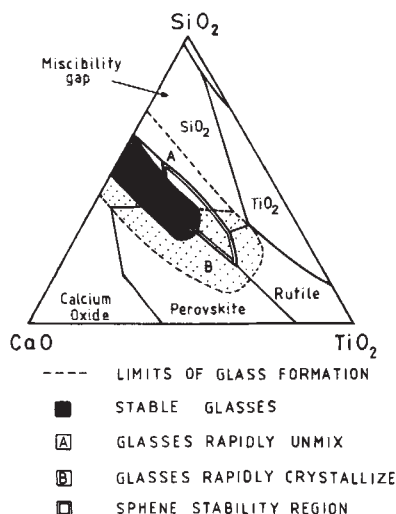


Fig. 1 Regions of glass formation in the SiO_2 - CaO - TiO_2 system, with the crystalline sphene stability field overlaid (from ref. 4).

Both XPS and SIMS results indicate some silica enrichment at the glass surface, believed to have been introduced from SiC used in polishing the disks. Polishing was finished with a diamond paste on a lead wheel and traces of lead are also observed on unleached samples. Apart from this, XPS reveals no significant surface contamination. XPS spectra show strong enhancement of the Ti 2p peak with increasing leach time, coupled with a sharp decline in the Ca 2p and Si 2p intensities. The element ratios $n_{\text{Ca}}/n_{\text{Ti}}$ and $n_{\text{Si}}/n_{\text{Ti}}$ as a function of leaching time, are summarized in Fig. 2. The larger than theoretical $n_{\text{Si}}/n_{\text{Ti}}$ values are partially due to non-stoichiometry at the glass surface for the reason mentioned above. The balance can be attributed to instrumental sensitivity differences between calcium and silicon, and is not observed in the SIMS results (Fig. 3).

Within 12 h leaching, a layer largely depleted in calcium and silica has been formed at the glass surface, at least to the maximum photoelectron escape depth (5–7 nm). SIMS results show this leached layer reaches depths of 40–50 nm after 24 h of leaching (Fig. 3). After 8 days leaching, XPS again shows a surface heavily depleted in calcium and silica. The SIMS profile indicates the layer extends much further into the glass, to a depth of at least 200 nm.

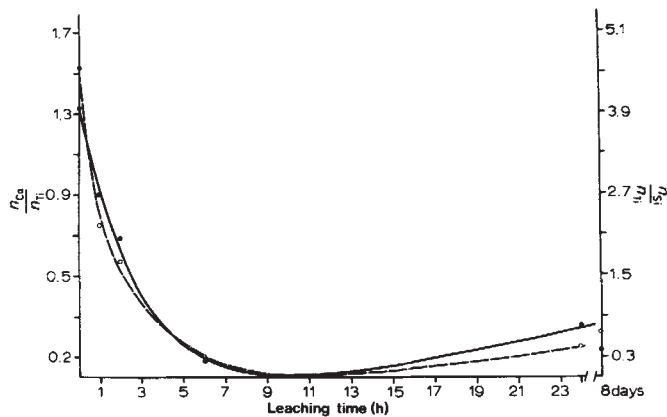


Fig. 2 Element ratios $n_{\text{Ca}}/n_{\text{Ti}}$ and $n_{\text{Si}}/n_{\text{Ti}}$ plotted against leaching time from XPS spectra of CaTiSiO_5 glasses leached in deionized water, 90 °C.

The 8-day SIMS profile differs significantly from earlier samples in a number of respects: the sputtering rate is considerably higher, indicating a material of lesser density, also the composition of the layer shows little variation through the entire sputtered profile, extending to more than three times the depth of profiles on earlier samples. Ti is enriched over Ca and Si by at least an order of magnitude and this is unchanged through 150 nm of sputtering. These observations suggest a precipitated layer, rather than a selectively leached layer as is observed on samples after 1–24 h of leaching. Examination of the 8 day sample by scanning electron microscopy (SEM) and energy dispersive X-ray analysis confirmed the presence of a surface coating several micrometres thick and rich in titanium. X-ray diffraction indicated the material was poorly crystalline TiO_2 (anatase). This implies either crystallization from the titanium-rich leached layer or precipitation of TiO_2 from the leachant. SEM and SIMS evidence described above, favours the latter.

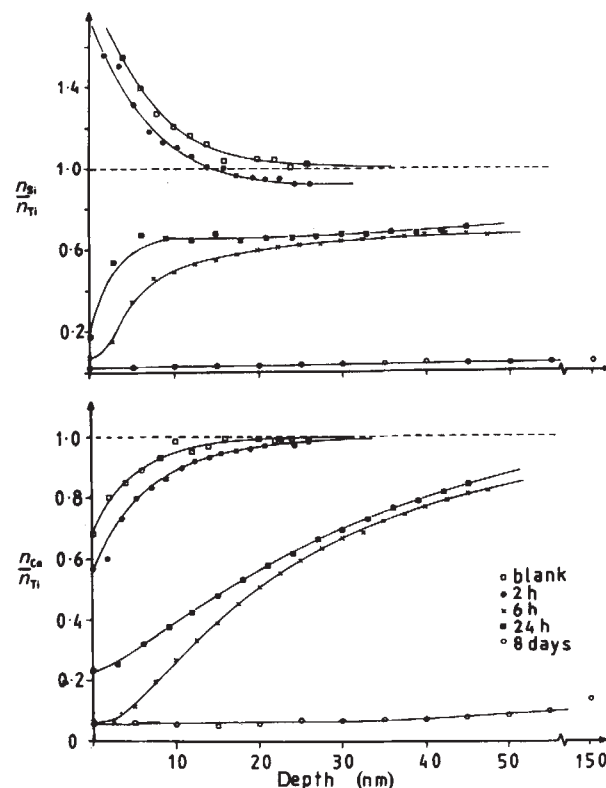


Fig. 3 Element ratios $n_{\text{Ca}}/n_{\text{Ti}}$ and $n_{\text{Si}}/n_{\text{Ti}}$ plotted against leaching time from SIMS profiles of CaTiSiO_5 glasses leached in deionized water, 90 °C.

Thus the leaching of a CaTiSiO_5 glass in deionized water appears to parallel the leaching of borosilicate glasses⁷⁻⁹, with a titania-rich rather than a silica-rich, alkali-deficient layer formed at the glass surface. Indeed in the initial stages of the leaching of the sphene glass, silica is leached almost as rapidly as calcium. Leach rates of crystalline and ceramic sphenes are similar to those of the glass in both magnitude and time dependence¹⁰. Also, such high-titanium coatings have been observed by SEM/EDX on crystalline sphene samples. This suggests common rate-controlling processes in all three. Differences would be anticipated in where attack is localized because of the surface properties of the polycrystalline materials compared with the relatively homogeneous surface of the glass.

The proposed leaching mechanism of titanates⁵, involving formation of a titanium dioxide gel layer of low solubility, is thus supported in these experiments with a titanosilicate glass. Such a layer is formed extremely rapidly, as revealed in the XPS results. Despite this TiO_2 layer, after 8 days the leach rate of these glass samples has only reached $\approx 10^{-9} \text{ kg m}^{-2} \text{ s}^{-1}$ (ref. 10), comparable to resistant borosilicates but still short of the performance of rutile encapsulated wasteforms³. This implies the TiO_2 layer does not provide a completely coherent protective layer: either the layer is relatively permeable, or it does not cover the entire surface. In either case, from the steady decline in leach rate observed, it is clear that the TiO_2 -rich layer inhibits leaching of the bulk glass, an important feature in assessing the long term durability of these materials.

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Sulphur and sulphate from Mt Erebus

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Although sulphur is the most common element in the Antarctic aerosol, no more than 6% of the sulphate can be of direct marine origin¹. Cadle *et al.*² and Bigg³ found much of the aerosol in the Antarctic to be a complex, partially neutralized, acid sulphate, although essentially 'pure' H_2SO_4 and $(\text{NH}_4)_2\text{SO}_4$ particles were common. Efforts to analyse size-segregated aerosol samples chemically have been generally unsuccessful. There is, therefore, a question of the origin of the sulphate component of the Antarctic aerosol. I consider here the contributions that Antarctic volcanic activity may make to the sulphate content of the Antarctic aerosol.

The Antarctic aerosol generally exhibits a bimodal size distribution with a nucleus mode centred near $0.01 \mu\text{m}$ diameter, which is apparently composed of freshly produced sulphate aerosol, and an accumulation mode centred between 0.2 and $1.0 \mu\text{m}$ (ref. 4). Shaw⁴ emphasizes the uncertain nature of the aerosol in the accumulation mode, which contains most of the aerosol mass, when he suggests the following list of origins (in order of importance): unidentified sources of sulphate particles,

local oceanic sources, southern hemispheric deserts, extra-terrestrial sources and exposed Antarctic surfaces. The oceans may also be a source of sulphate aerosol indirectly through the oxidation of biogenic sulphur gases such as dimethyl sulphate or CS_2 (refs 5-8).

While in the Arctic a significant fraction of the aerosol is pollution-transported from mid-latitudes⁹, the Antarctic aerosol shows little perceptible anthropogenic influence. Thus, Boutron and Delmas¹⁰, in reviewing measurements of heavy metals and sulphur compounds in Antarctic ice cores, could find no evidence for anthropogenic influences during the last century. The only significant modulation in the concentrations of these constituents appeared to be due to vulcanism, including Antarctic volcanoes. They noted that the aerosol in the Antarctic appears to be 'enriched' with heavy metals, compared to typical crustal concentrations, but they suggested that this was probably due to a natural (possibly biological) process rather than to anthropogenic activity. However, these conclusions with respect to Pb have been disputed by Ng and Patterson¹¹ who argue for a greater anthropogenic influence.

Mt Erebus, a 3,794-m composite volcano on Ross Island, is the only Antarctic volcano that is currently emitting significant amounts of trace gases and particles south of 63° latitude¹². (Recently, Gonzalez-Ferran¹³ has reported sighting another 'steaming' low basaltic volcanic cone some 200 km east of Palmer Station, Antarctica, but its emission characteristics are unknown.) The crater of Mt Erebus is persistently filled with magma and numerous active fumaroles and vents are found throughout the crater area. Mt Erebus is noted for its lava lake and long periods of minor strombolian eruptive activity. Ross noted its extended plume in 1841, as did Scott in 1903¹⁴. However, the volcanic activity of Mt Erebus is quite variable¹⁵. During the period of our observations, in November 1980, the plume from Mt Erebus was visible on every fine day and sometimes could be seen extending up to 100 km from the mountain.

The measurements to be described were made aboard the Lockheed LC-130R aircraft. Optical particle counters aboard this aircraft provided measurements of atmospheric particle size distributions over the size range 0.09 – $11 \mu\text{m}$ (these single particle counters provided measurements in 75 size intervals over the sizing range), and the total number concentrations of particles in the air were measured with an Aitken nucleus counter modified for background monitoring¹⁶. The light scattering coefficient due to particles was measured at three wavelengths with an integrating nephelometer¹⁷. The concentrations of gaseous sulphur in the emissions from Mt Erebus were measured with a flame photometric detector; sulphur gas scrubbers, which selectively removed SO_2 and H_2S , were used to detect the concentrations of SO_2 and H_2S . Particles were also collected on filters for later bulk chemical analysis by means of ion chromatography.

The measuring system is shown in Fig. 1. As the aircraft was pressurized, provision was made for a nearly isokinetic entry of air into a bag sampler. Once filled, the bag sample was pressurized to cabin pressure by filling the outer pressure vessel with filtered air from the cabin, thus partially collapsing the bag. By this means, an air sample at cabin pressure was provided to the two optical particle counters. Before sizing, the particles were dried to below 40% relative humidity by passing them through a diffusion dryer which effloresced any deliquescent particles to near their 'dry' diameters. Sampling, data processing, display and recording were controlled automatically by a minicomputer.

These specialized measurements were augmented by devices for measuring the meteorological state parameters, air motion sensors, an inertial navigator and a data recorder. Data from this system were used to place the particle and gas measurements in geographical and meteorological contexts.

To obtain quantitative estimates of the fluxes of particles and gases from Mt Erebus, the aircraft repeatedly traversed the plume of effluents from top to bottom in vertical sections normal

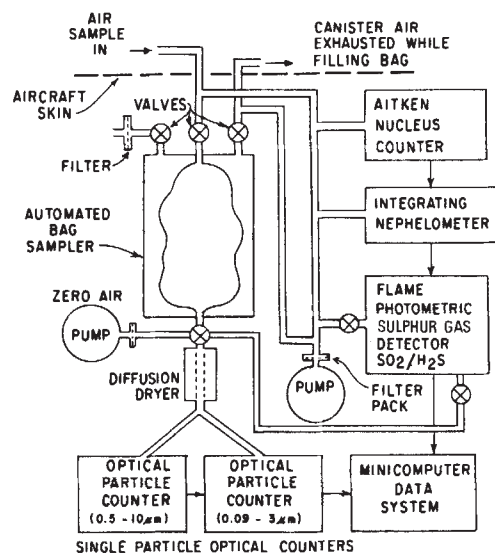


Fig. 1 Schematic of the particle measuring instrumentation aboard the LC-1300R aircraft.

to the axis of the plume. The dimensions of the plume were determined from continuous measurements of either Aitken nuclei or sulphur gases. Measurements of these parameters were also used to trigger automatically bag sampling of the effluents near the plume centre. The sulphur gas analyser could sample either from the bag or from the free air stream. Concentrations so determined, together with the plume cross-sectional area and the wind speed (measured by the aircraft inertial navigator), yielded the flux of various components. This technique has been successfully used in other volcanic plumes^{18,19}. With steady-state emissions and moderate winds, it yields good results in a neutral or stably stratified atmosphere.

Airborne measurements of the effluents in the plume from Mt Erebus were obtained on 5, 7, 8 and 14 November 1980. High winds and turbulence reduced the data quality on 5 November, and on 7 November they caused the plume to descend down the side of the volcano, rendering flux estimates impossible. However, on 8 and 14 November, when the winds were lighter and terrain-induced turbulence was minimal, an extensive series of plume penetrations was made, and significant masses of volcanic aerosol were collected on the filters. The results of the measurements obtained on 14 November are described below.

On 14 November the plume from Mt Erebus was generally white as it emerged from the crater. After a few hundred metres of travel it acquired a distinct bluish cast when viewed against the sky. When the plume was viewed by transmitted light from cloud or snow cover, it was nearly invisible, except when viewed along the axis of the plume, when it was yellowish-white.

The plume coloration was evidently due to most of the aerosol being small compared with the wavelength of visible light²⁰.

Measurements of the size distribution of particles in the plume (Fig. 2) confirm this deduction. The major features revealed by these distributions were seen on all of the flights. Virtually all of the aerosol volume (mass) in the plume is narrowly confined to particles $\sim 0.1 \mu\text{m}$ diameter (this size region is well resolved by 11 size intervals and excellent counting statistics). For particle sizes $\geq 1 \mu\text{m}$, the plume and ambient samples are indistinguishable, both having aerosol volumes of $2\text{--}3 \mu\text{m}^3 \text{cm}^{-3}$. If the average particle density was similar to that for $(\text{NH}_4)_2\text{SO}_4$ or H_2SO_4 , this corresponds to an aerosol mass of $\sim 4\text{--}6 \mu\text{g m}^{-3}$. In contrast, the sub-micrometre volcanic aerosol mass was $\sim 90 \mu\text{g m}^{-3}$, compared with $\sim 1 \mu\text{g m}^{-3}$ for the ambient air.

We have not previously observed such narrow particle size distributions in a volcanic plume, although high concentrations of particles $< 0.1 \mu\text{m}$ are common in SO_2 -rich post-eruptive volcanic plumes²¹. These small particles are usually due to gas-to-particle conversion within the vents and in the plume and to the products left behind after droplets evaporate. The narrow particle-size distribution in the plume of Mt Erebus suggests that a single mechanism dominated the aerosol production and that most of this production took place near the crater and immediately downwind. The primary removal mechanisms for particles of this size would be through cloud and precipitation scavenging. However, these particles should be exceptionally long lived as the peak in their mass distribution is located within the so-called 'Greenfield Gap', the scavenging efficiency minimum^{22,23}.

A filter sample was exposed over a large portion of the plume cross-section 13 km downwind of Mt Erebus on 14 November (at the flow rates used the filter media, a Ghia Corp. 37 mm stretched Teflon filter, removed virtually all of the particulate). Ion chromatographic analysis of the water-soluble anions on this filter showed Cl^- ($5.2 \mu\text{g m}^{-3}$), SO_4^{2-} ($9.4 \mu\text{g m}^{-3}$) and NO_2^- ($2.2 \mu\text{g m}^{-3}$), all present in concentrations well above ambient values. In fact, these three anions (assuming a reasonable cation mix) made up much of the aerosol mass determined from the size distribution measurements. (This observation also places a limit on the maximum emission of the so-called 'volcanic enriched elements'.)

Estimates of the emission fluxes of various materials from Mt Erebus are shown in Table 1, where they can be compared with those from some other volcanoes. The data for White Island, St Augustine, Kverkfjöll and St Helens were taken using instruments and techniques similar to those used in the present study. The Mt Etna data were deduced by Zettwoog and Hauget²⁴ from correlation spectrometer measurements. The measurements of Faivre-Pierret (unpublished report, see ref. 12) and Polian and Lambert¹² were obtained using treated filters at the crater rim and visual estimates of the velocity of the vent emissions.

The sulphur gas emissions from Mt Erebus were almost entirely SO_2 . Our estimates of the SO_2 flux emissions range from 0.4 to 1.8 kg s^{-1} (or an average of $4 \times 10^4 \text{ ton yr}^{-1}$ expressed as sulphate). The corresponding particle fluxes ranged from 6.6×10^{15} to $3.4 \times 10^{16} \text{ s}^{-1}$. Using the measured plume characteristics for 14 November, the sulphate aerosol

Table 1 Emission fluxes from Mt Erebus compared with post-eruptive emissions from other volcanoes

Volcano	Date	Flux of SO_2 (kg s^{-1})	Flux of SO_4^{2-} (kg s^{-1})	Total flux of particles (s^{-1})	Ref.
Mt Erebus (Antarctica)	5 November 1980	1.80		3.4×10^{16}	This work
	8 November 1980	0.45		1.2×10^{16}	This work
	14 November 1980	0.40	6.0×10^{-2}	6.6×10^{15}	This work
	1978	0.019			R. Faivre-Pierret unpublished report
	1978	0.020	$\sim 10^{-3}$		12
White Island (New Zealand)	15 October 1980	1.75		4.0×10^{18}	This work
St Augustine (Alaska, USA)	22 April 1977	0.3		6.0×10^{15}	19
Mt Etna (Italy)	May 1979	12–14			24
Kverkfjöll (Iceland)	25 June 1980	0.03		6.0×10^{14}	This work
Mt St Helens	September–December 1980	0.1–40	0.1–1	$4 \times 10^5\text{--}10^{17}$	29

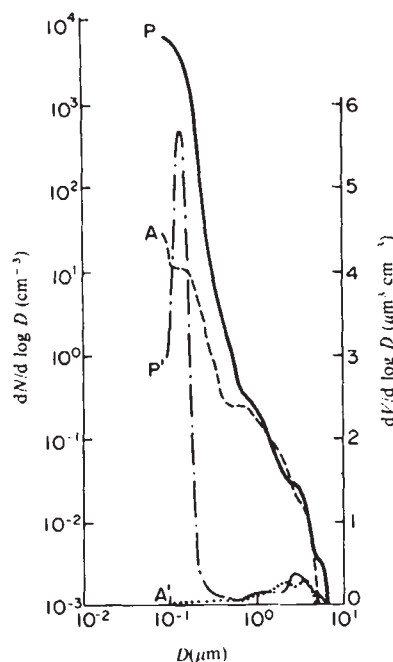


Fig. 2 Particle number (P) and volume (P') distributions in the plume of Mt Erebus at 13 km downwind of the mountain and at 4.5 km pressure altitude on 14 November 1980. Corresponding curves for the ambient are shown by A and A'.

flux, derived from the filter samples, was estimated at 0.06 kg s^{-1} (or $2 \times 10^5 \text{ ton yr}^{-1}$).

Our estimates of the SO_2 emission fluxes from Mt Erebus are substantially greater than those estimated by Faivre-Pierret and Polian and Lambert¹². Polian and Lambert¹² estimated from their measurements that $\sim 10^3 \text{ ton yr}^{-1}$ of sulphate ($\text{SO}_2 + \text{particulate SO}_4^{2-}$) is emitted by Mt Erebus. As the estimated total sulphate budget for Antarctica is $1.3\text{--}2 \times 10^5 \text{ tons yr}^{-1}$ (ref. 3, and C. Boutron, personal communication), Polian and Lambert¹² concluded that the emissions from Mt Erebus were unimportant in the sulphate budget of Antarctica. Obviously this conclusion must be re-examined in the light of our measurement of total sulphate emissions from Mt Erebus of $0.42 \times 10^5 \text{ ton yr}^{-1}$. If our measurements are representative, then as much as 32% of the Antarctic total sulphate budget could be provided by Mt Erebus. Clearly, however, further measurements of the emission rates of sulphur and particles from Mt Erebus are needed.

In view of the geographical and meteorological settings of Mt Erebus, its emissions could have an impact on the whole continent. Long-range transport is assured by the elevation of the source (3,794 m), the small size (and therefore low settling speed and minimum scavenging efficiency) of the directly emitted aerosol ($0.1 \mu\text{m}$), and the fact that the bulk of the sulphur is emitted as SO_2 . [It must be emphasized that the total emission of SO_2 (as sulphate) is ~ 25 times greater than the direct sulphate emission and thus it is the behaviour of sulphur gas which is of primary interest.] The SO_2 should be converted slowly to sulphate; for example, Hobbs *et al.*²⁹ measured a gas-to-particle conversion rate of $\sim 0.1\% \text{ h}^{-1}$ in the fumarolic volcanic emissions from Mt St Helens. Thus, sulphate deposition in the immediate region of Mt Erebus should be small, and limited to occasional, inefficient, precipitation scavenging of the directly emitted sulphate particles. The role of low removal rates in promoting long-range transport of Antarctic aerosol is supported by the observations of Shaw²⁵ who suggests that the sulphate aerosol residence time may be >30 days. Most of the sulphur gases and sulphate should follow the poleward drift of the cyclonic storms as they are driven around the continent by the circumpolar vortex²⁶. The poleward flow subsides at the pole and is balanced, in part, by the thermal gradient wind flowing off the ice-bound continent in the lower troposphere. Hogan^{27,28}

cites evidence for the transport of aerosol from the continental margins to the surface at the South Pole by such a route.

Once the sulphur is brought down to the lower troposphere by polar subsidence, it should be distributed radially by the gradient wind. The combination of slow gas-to-particle conversion, poleward transport, subsidence and radial distribution, and subsequent removal by surface deposition and ice particle scavenging, could result in the sulphur emissions from Mt Erebus affecting the Antarctic sulphate on a continental scale.

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¹⁰Be profiles in seawater

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A knowledge of the marine geochemical cycle of ¹⁰Be is basic to the use of this long-lived cosmogenic radioisotope (half life 1.5 Myr) in geochronological and geophysical studies^{1–3}. The first attempts at measuring ¹⁰Be in seawater were made only recently using nuclear accelerator spectrometry, and six concentration values from four locations have been reported^{4–6}. This data base must be expanded to include detailed water column profiles, so that the behaviour of ¹⁰Be in the ocean can be more fully described. A parallel case in point is the recent measurement⁷ of a detailed oceanic profile for the stable ⁹Be. We report here two water column profiles of ¹⁰Be: one in the Drake Passage near Antarctica and the other in the San Nicolas Basin about 100 km off the coast of Southern California. The San Nicolas Basin profile clearly demonstrates the regulation of ¹⁰Be distribution by particulate cycling. The data confirm the earlier suggestion⁴ of particle uptake of ¹⁰Be in the surface ocean. They also furnish evidence for regeneration of ¹⁰Be across the thermocline and near the sea floor, and for mid-water scavenging.

Our analytical results are summarized in Table 1. The Drake Passage profile was obtained by combining waters taken from four stations (stations 32–35) occupied by RV *Hudson* during 1970. The latitudes of these stations ranged from 57°49' to 63°34' S, and the longitudes from 66°48' to 68°10' W. Approximately equal amounts of water from each station were used, and each depth interval represents a combination of about 10 samples from various depths within that interval.

Sampling in the central part of the San Nicolas Basin (33°00' N, 119°00' W) was carried out during a July 1981 cruise of RV *Velero IV*. The water depth there was 1,800 m and the basin sill depth ~1,100 m. Seven subsurface samples of ~100 litres each were collected from 20 to 1,500 m using 30-litre PVC Niskin bottles. Surface water at the same location was retrieved using the ship's salt water pump system. An additional surface water sample was obtained this way while the ship was in transit between San Nicolas Basin and Los Angeles Harbor. This last sample was passed through a 0.4- μ m pore-size Nucleopore filtration system directly attached to the ship's pump. All samples were acidified with H₂SO₄ to pH ~2 and stored in plastic containers on board.

In the laboratory, Fe³⁺ and ⁹Be (4.2 mg BeSO₄ or 2.55 $\times$ 10¹⁹ Be atoms) carriers and a ⁷Be tracer were added to the acidified water. After addition of ammonia to form iron hydroxide, a saturated Na₂CO₃ solution was added to effect CaCO₃ precipitation which facilitated settling of the iron hydroxide. The precipitates were taken up with HCl and any insoluble matter present was removed by filtration (no ⁷Be was found on the filter). Subsequent procedures for extraction of Be followed that given in ref. 8, with minor modifications. The recovery yield as checked by ⁷Be varied from 43 to 98%, averaging 79%. ¹⁰Be was measured using the McMaster University model FN tandem Van de Graaff accelerator⁹. Reagent blanks submitted to the identical treatment as the unknowns were measured at the same time.

We have found that the ¹⁰Be concentration measured in blanks was mainly due to the ⁷Be tracer used (Amersham

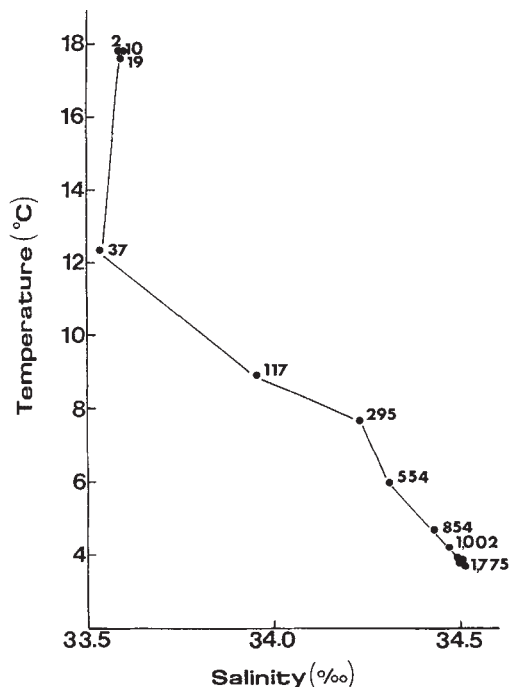


Fig. 2 Temperature-salinity diagram of San Nicolas Basin. Water depths (m) are shown (data provided by D. E. Hammond).

BSG.1, lot 56) (in retrospect we realize that it would have been preferable not to use the tracer, as it is not required for the ¹⁰Be analyses). The blank corrections are fairly large and render the uncertainties quoted for ¹⁰Be concentrations considerably higher than those of the measured ¹⁰Be/[⁹Be carrier] ratio (Table 1). However, most of the results do still carry precisions of <20%.

The Antarctic waters had been stored unacidified in high-density linear polyethylene 'jerri-jugs' for >10 yr before analysis. The affect of this long storage on their ¹⁰Be contents is unknown; thus too much confidence cannot be placed on the Drake Passage data: they can at least be taken as lower limits, however. The rather uniform water-column distribution of ¹⁰Be and the high ¹⁰Be concentration in the surface layer (as opposed to those of the low-latitude seas^{4,5}) appear consistent with the intense vertical mixing characteristic of this part of the ocean¹⁰. An analogy can be found in the case of ²²⁶Ra (ref. 11).

Much more can be said about the San Nicolas Basin profile. Figure 1 shows that the ¹⁰Be concentration at surface is comparable to or slightly higher than those reported for the open ocean^{4,5}. It falls rapidly to a minimum in the vicinity of 20–50 m (one would refrain from placing too much emphasis on the data point at 20 m because of its very large analytical error). Increasing downward through the thermocline, it reaches a constant value of close to 1,200 atoms g⁻¹ between 500 and 1,000 m. Below this depth there is indication of further increase towards the bottom. Thus except at the very surface and near the bottom, the vertical distribution resembles those of P and Si (Fig. 1). Although the locations of the two surface samples do not coincide exactly, their similar ¹⁰Be contents (Table 1) suggest that ¹⁰Be in the ocean exists mainly in soluble form (<0.4 μ m). This is consistent with Silker's observation¹² that <10% of the ⁷Be activities in surface seawater is in the particulate fraction.

Surface input of ¹⁰Be accompanied by active removal must have taken place in the upper 100 m above the thermocline. The approximate rates of these processes can be estimated as follows. Consider the stationary condition in the vertical dimension. If we place the ¹⁰Be minimum at 50 m (mid-point of the layer), we can crudely calculate the downward (0 $\rightarrow$ 50 m) and upward (100 $\rightarrow$ 50 m) diffusive fluxes of ¹⁰Be along concentration gradients. Taking a vertical diffusivity (*K*) of 1.6 cm² s⁻¹ as derived from the ²²⁸Ra distribution in the surface

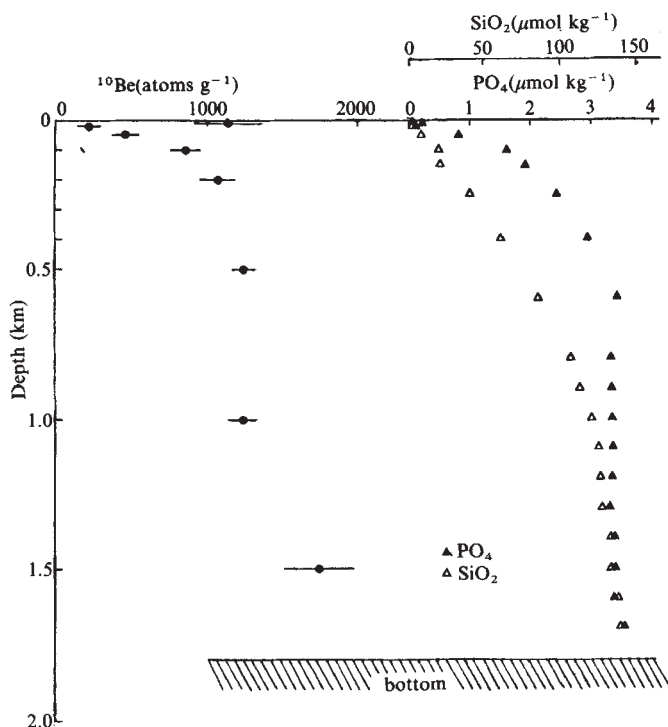


Fig. 1 Water-column ¹⁰Be profile in San Nicolas Basin. Comparison with the phosphate and silicate distributions (data provided by D. E. Hammond) suggests a biogenic origin for the ¹⁰Be-carrying particles and that ¹⁰Be, like P or Cu, could be associated more with the 'tissue' part than the 'hard' part of such particles.

Table 1 Measurement of ^{10}Be in seawater

Water depth (m)	Sample weight (kg)	$^{10}\text{Be}/[^9\text{Be carrier}]$ ($\times 10^{-12}$)	$^{10}\text{Be conc.}^*$ ($10^3 \text{ atoms g}^{-1}$)
San Nicolas Basin			
0†	62.3	4.7 ± 0.5	0.9 ± 0.2
0	100.5	7.1 ± 0.9	1.1 ± 0.2
20	93.3	3.4 ± 0.3	0.2 ± 0.1
50	89.3	4.2 ± 0.3	0.5 ± 0.1
100	97.4	5.9 ± 0.4	0.9 ± 0.1
200	88.2	6.3 ± 0.4	1.1 ± 0.1
500	92.4	7.1 ± 0.3	1.2 ± 0.1
1,000	97.4	7.3 ± 0.4	1.2 ± 0.1
1,500	85.2	8.4 ± 0.8	1.7 ± 0.2
Blank	—	2.6 ± 0.3	—
Drake Passage‡			
5–250	161.2	10.4 ± 2.0	1.4 ± 0.3
290–990	143.9	8.9 ± 0.3	1.3 ± 0.1
1,100–2,000	123.8	8.8 ± 0.6	1.4 ± 0.1
2,500–3,700	144.8	10.5 ± 0.6	1.5 ± 0.1
Blank	—	1.8 ± 0.2	—

* Errors include those of the blanks.

† Nucleopore (0.4 μm) filtered.‡ ^{10}Be concentration values may represent lower limits (see text).

water of this region¹³, and assuming linear gradients, the downward and upward fluxes are 6.9×10^6 and 4.0×10^6 atoms $\text{cm}^{-2} \text{yr}^{-1}$, respectively. These fluxes are to be balanced by particulate removal. As there are 7.3×10^6 atoms cm^{-2} in this surface layer, the mean removal time (τ) is about 8 months. This could be an upper-limit estimate as we have neglected any advective transport from below. We may also evaluate τ using a simple box model⁴ of the relationship $\tau = A/(Q + wC_d)$, where A is the abundance in the 100 m water column (7.3×10^6 atoms cm^{-2}), Q is the surface input (6.9×10^6 atoms $\text{cm}^{-2} \text{yr}^{-1}$), C_d is the average deep-water ^{10}Be concentration (1.2×10^3 atoms cm^{-3}), and w is the upwelling rate. Fitting the deep-water (500–1,800 m) temperature data to a diffusion–advection model¹⁴ gives a scale height (K/w) of 400 m. The near-bottom water in the basin has a K value of $17 \text{ cm}^2 \text{s}^{-1}$ (ref. 15). Therefore w becomes 134 m yr^{-1} , giving $\tau \approx 4$ months. This estimate tends to be minimal because the upwelling rate at the base of the thermocline could well be smaller than near the bottom. The mean residence time with respect to particle scavenging for ^{10}Be in the mixed layer is thus of the order of 0.5 yr, which is comparable with that of the rather reactive isotopes of Th in the area¹³.

The surface input value of 6.9×10^6 atoms $\text{cm}^{-2} \text{yr}^{-1}$ of ^{10}Be deduced here is 5–10 times higher than the estimated atmospheric flux^{16,17}. We do not have a definite explanation for this difference. One possible candidate could be ^{10}Be input from river. River water contains ^9Be 2–3 orders of magnitude higher than surface seawater^{7,18}. Could it also have high ^{10}Be concentration? Raisbeck *et al.*¹⁹ reported relatively high ^{10}Be concentrations of $15\text{--}73 \times 10^3$ atoms g^{-1} in precipitations and of 7.3×10^3 atoms g^{-1} in a River Seine water sample in France. As shown in Fig. 2, the surface low salinity, a common feature of the California Current, does reflect the discernible fresh water component. The presence of riverine ^{10}Be would seem plausible, although this conjecture needs to be verified by future work. Note that the surface input of 6.9×10^6 atoms $\text{cm}^{-2} \text{yr}^{-1}$ is consistent with the ^{10}Be deposition rate of 6.5×10^6 atoms $\text{cm}^{-2} \text{yr}^{-1}$ in the sediment; the sediment in this area has been found to accumulate at $13 \text{ mg cm}^{-2} \text{yr}^{-1}$ (ref. 20) and to contain an authigenically precipitated ^{10}Be concentration of about 5×10^8 atoms g^{-1} (ref. 21). This adds to the validity of the steady-state assumption used in our discussion.

The remainder of the water-column ^{10}Be distribution, as in the case of ^9Be recently reported for an open ocean station in the Pacific⁷, depicts thermocline and sea-floor regeneration and mid-water scavenging. Below ~ 500 m, temperature (T) and salinity (S) in the basin are linearly related (Fig. 2). A similar plot of T against ^{10}Be shows a concave-up pattern indicating

in situ particulate scavenging coupled with a bottom source of ^{10}Be . The P and Si data do not exhibit such features, as can also be discerned in Fig. 1. The bottom source strength, estimated from the diffusivity and concentration gradient, is 7.3×10^6 atoms $\text{cm}^{-2} \text{yr}^{-1}$. This is as large as the net downward flux through the thermocline, which should equal the surface input of 6.9×10^6 atoms $\text{cm}^{-2} \text{yr}^{-1}$. As we have done for the surface layer, from these two inputs into the deep water below 100 m which has a ^{10}Be standing crop of 2×10^8 atoms cm^{-2} , we obtain a deep-water scavenging residence time of 14 yr (one may also obtain values of 4–28 yr applying the advection–diffusion model of Craig²² to depths below 500 m; the range reflects the error limits associated with the three deep-water data points). This compares with the ultimate ^{10}Be residence time in the basin of 30 yr (=standing crop of whole water column/surface input), and implies that an average ^{10}Be atom after deposition is recycled back once more to the overlying water before ultimate removal.

We have examined the vertical distribution of ^{10}Be in seawater in sufficient detail to assess semi-quantitatively the scavenging and regeneration rates in the water column. The residence times of ^{10}Be in the San Nicolas Basin are estimated as ranging from 0.5 yr in the surface layer to 30 yr in the basin as a whole. These estimates are probably lower limits for the average oceanic condition, as the San Nicolas Basin is high in surface productivity and particle fluxes compared with the open ocean²⁰. Nonetheless, they do point to the relatively high reactivity of ^{10}Be , and support the contention⁵ that the oceanic residence time will not be a limiting factor for recovering ^{10}Be production variations in marine sediments down to the time scale of oceanic mixing ($\sim 10^3$ yr). Given the possibility that the mean residence time of ^{10}Be could be shorter than the ocean mixing time, the extent of tracer equilibrium between ^{10}Be and its stable counterpart ^9Be in the sea needs to be studied, as it bears relevance to ^{10}Be marine geochronology⁷. Simultaneous measurements of ^{10}Be and ^9Be in seawater are being carried out.

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Early Proterozoic climates and plate motions inferred from major element chemistry of lutites

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The early Proterozoic Huronian Supergroup of the north shore of Lake Huron (Fig. 1) is a thick (up to 12,000 m) succession of sedimentary and volcanic rocks deposited between about 2,500 and 2,100 Myr ago¹. Here we present a palaeoclimatic interpretation of the Huronian based on approximately 200 major elements analyses of lutites. Most of these are new analyses from the Gowganda and Serpent Formations (Fig. 2). The remainder are from published sources cited in Fig. 4. The composition of lutites from the Huronian Supergroup records an early period of intense, probably tropical, weathering followed by climatic deterioration that culminated in widespread deposition of glaciogenic sediments of the Gowganda Formation. Climatic amelioration followed during deposition of the succeeding Huronian formations. The Huronian succession can be interpreted using a uniformitarian approach in that present day seafloor spreading rates and latitude-related climatic variations are compatible with available geochronological and palaeomagnetic data.

Earlier studies of the palaeoclimatology of Huronian rocks have largely revolved around the Gowganda Formation which has been interpreted both as glacial or glaciomarine and/or as fault-related submarine debris flows²⁻⁴. Diamictites of the Bruce and Ramsay Lake Formations (Fig. 2) have also been considered to be glaciogenic⁵. It has been suggested that pre-Huronian palaeosols and pyritic uraniferous conglomerates at the base of the Huronian formed in a cold climatic regime^{6,7}. Gay and Grandstaff⁸ noted that palaeosols developed beneath the Huronian appear to represent intense weathering and suggested a temperate humid regime.

The 'aluminous' nature of the Pecors Formation led Roscoe⁶ to conclude that a regolithic cover was restored on the landmass following deposition of the possibly glaciogenic Ramsay Lake Formation. Dolomite in the Espanola Formation has been interpreted as indicating an amelioration of climate after deposition of the glaciogenic(?) Bruce Formation.

Following the major glacial period of Gowganda deposition, tropical-type weathering conditions have been inferred from the presence of diaspore, kaolinite, pyrophyllite, andalusite and kyanite in the overlying Lorrain Formation⁹⁻¹¹. Temperate conditions have been suggested¹² during deposition of the Gordon Lake Formation and the presence of gypsum and anhydrite¹² may indicate a somewhat warm and dry climate. Quartzites of the Bar River Formation contain kaolinite and pyrophyllite which were interpreted¹² as indicating tropical weathering conditions. Card¹³ stated that rocks of the lower part of the Huronian are immature and indicate negligible weathering of the source rocks. He contrasted this with the upper Huronian which contains evidence of extreme chemical alteration of feldspars.

There is, therefore, no consensus on palaeoclimatic interpretation of Huronian rocks although most authors accept a glaciogenic origin for the Gowganda Formation and possibly also for other diamictites lower in the Huronian succession. We use a geochemical approach in an attempt to resolve some of these problems. Mineralogical evidence from the upper Huronian formations, above the Gowganda Formation, is consistent with a high degree of chemical weathering. Apart from the glaciogenic(?) diamictites, the rocks of the lower part of the Huronian Supergroup have been variously interpreted as

having formed in a frigid climate or in temperate humid conditions.

Wedepohl¹⁴ estimated that the upper crust consists of approximately 21% by volume of quartz, 41% plagioclase and 21% potassium feldspar. Feldspars are by far the most abundant of the reactive (labile) minerals; consequently the dominant process during chemical weathering of the upper crust is the degradation of feldspars and concomitant formation of clay minerals. Calcium, sodium and potassium generally are removed from the feldspars by aggressive soil solutions so that the proportion of alumina to alkalis typically increases in the weathered product. A good measure of the degree of weathering can be obtained by calculation of the chemical index of alteration (CIA) using molecular proportions:

$$CIA = [Al_2O_3 / (Al_2O_3 + CaO^* + Na_2O + K_2O)] \times 100$$

where CaO^* is the amount of CaO incorporated in the silicate fraction of the rock. A correction is made for carbonate and apatite content. The resultant value is a measure of the proportion of Al_2O_3 versus the labile oxides in the analysed sample. Values of the index are 50 for unaltered albite, anorthite and potassic feldspars, whereas the value for diopside is 0; consequently fresh basalts have values between about 30 and 45 and granites and granodiorites have higher values, ranging between 45 and 55. Idealized muscovite gives a value of 75 and illite ranges between 75 and 85 as do montmorillonites and beidellites. Kaolinite and chlorite yield the highest values, very close to 100. Thus values for average shales range from about 70 to 75 (Fig. 4) because of the large proportion of clay minerals.

Figure 3 is a plot of CIA values against depth in two fairly mature Recent weathering profiles developed on a basalt in southeastern Australia¹⁵ and a granite from Guyana¹⁶. There is an obvious trend from low values in fresh rock to progressively higher values in more intensely altered materials. The changes in CIA reflect changes in the proportion of feldspar and the various clay minerals in the profiles. Such profiles ultimately are subject to mass wasting, the materials being transported and deposited in sedimentary basins as sands and muds. Size sorting during transportation and deposition generally results in some degree of mineralogical differentiation¹⁷ which may modify the CIA. We have therefore restricted this study to rocks of mud grade (lutites). The mineralogical and chemical composition of the muds will reflect the intensity of weathering

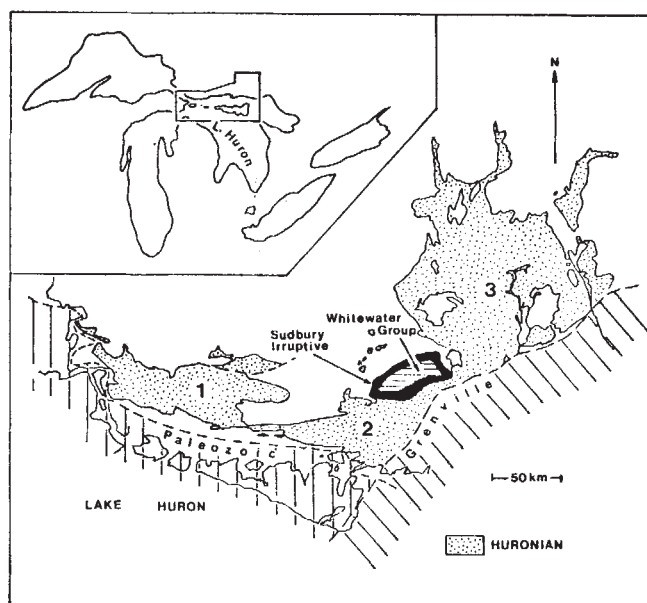


Fig. 1 The distribution of the early Proterozoic Huronian Supergroup. Rocks of area 1 and area 3 are generally less deformed and metamorphosed than those of area 2.

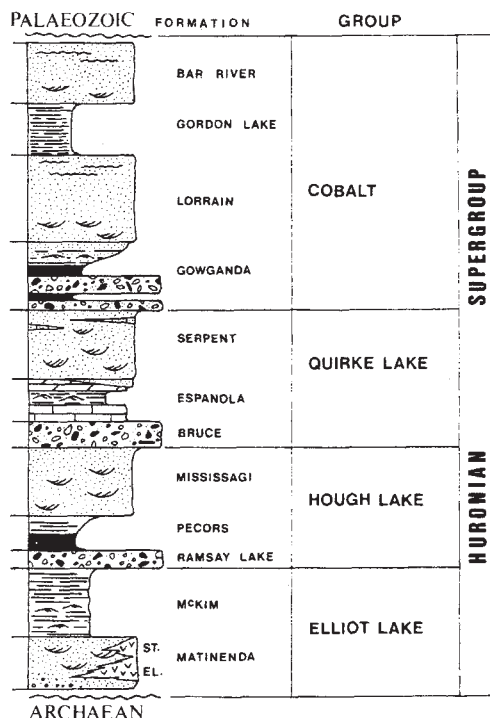


Fig. 2 Generalized stratigraphical column of the Huronian Supergroup. Note the presence of three major diamictite horizons (Ramsay Lake, Bruce and Gowganda Formations). Letters EL and ST near the base of the column represent the dominantly volcanic Elsie Mountain and Stobie Formations respectively. Maximum thickness of the succession is ~12,000 m.

because highly aluminous minerals such as kaolinites and beidellites are produced in quantity during intensive weathering and such sediments will have correspondingly high CIA values. Mass wasting of profiles in which chemical weathering is minimal, such as those produced in glacial conditions, may result in fine grained detrital sediment containing less aluminous clay minerals (montmorillonites and illites) and a high proportion of unaltered comminuted feldspar.

All of the analysed rocks have undergone some degree of metamorphism, ranging from 'sub-greenschist' facies in the north (areas 1 and 3, Fig. 1) to upper greenschist-amphibolite facies in the south¹⁸. The CIA values are not considered to have been significantly changed during metamorphism because similar values have been obtained from diamictite matrix materials of the Gowganda Formation from northerly areas of lower metamorphic grade and more highly metamorphosed rocks to the south (area 2, Fig. 1).

To facilitate interpretation of CIA values obtained from Huronian lutites, values were also calculated from a variety of other rocks and sediments. The average chemical composition of the Archean Shield of north-west Ontario¹⁹ with a value of 49.5 is included in Fig. 4 to give an idea of the Huronian source rocks^{20,21}. Chemical analyses of the fine grained portion of the matrix of nine Pleistocene till samples collected by R.W. Stewart in the southern part of the Archean Superior Province of the Canadian Shield yielded CIA values of about 52, close to the value obtained from the average shield composition. Data from Pleistocene glacial clays^{17,22} gave values in the 60–65 range. These are considerably lower than average shale values which plot in the 70–75 range (Fig. 4). The highest values shown in Fig. 4 were obtained from residual clays listed by Pettijohn¹⁷ and from sediments of the Amazon cone²³.

Fossil soils or palaeosols have been noted in several places beneath the Huronian succession^{6,7,8,24}. Near-complete removal of Ca and Mg, and the development of deep (>10 m) weathering profiles suggest fairly intense weathering under a wet or humid climate. The abundance of sericitic matrix material in sandstones of the Matinenda and Mississagi Formations indi-

cates that chemical weathering was both deep and intensive before the onset of Huronian deposition.

The names Elsie Mountain and Stobie Formation and other local formation names have been applied to thick dominantly volcanic units at the base of the Huronian succession¹³. These are probably equivalent in part to the Matinenda Formation as suggested in Fig. 2. Metamorphosed sedimentary rocks comprise about 10% of the Elsie Mountain Formation¹³. They are mostly thin intercalations in metamorphosed basalt flows. The Elsie Mountain Formation has yielded the highest CIA values of all the Huronian lutites examined (Fig. 4) suggesting a high degree of alteration by weathering processes.

The Stobie Formation also consists of interbedded metamorphosed volcanic and sedimentary rocks. CIA values obtained from lutites of the Stobie Formation yielded an average just greater than 70, comparable to the average shale.

The McKim Formation includes sandstones, siltstones and argillites. It varies greatly in thickness (up to 2,000 m in the east⁴) and appears to be largely resedimented. CIA values from fine grained rocks of the McKim Formation show a wide variety of values ranging from 67 to 86, with an average of about 75. The high values indicate the weathered nature of some of the materials incorporated in turbiditic mudstones of the McKim Formation. Lutites of the Elsie Mountain and McKim Formations include high CIA values comparable to residual clays cited by Pettijohn¹⁷ and sediments of the Amazon cone²³ (Fig. 4).

Lower CIA values from the Pecors and Serpent Formations suggest that, following deposition of the McKim Formation, there was a cooling climatic trend. Values for the Pecors Formation (average 71.5) are lower than those obtained from average shales. Mudstones from the upper part of the Serpent Formation in the southern part of the Huronian outcrop belt give still lower values.

Diamictites of the Ramsay Lake and Bruce Formations (Fig. 2) have been considered to be glaciogenic and may provide independent evidence of a cooling climatic trend beginning after deposition of the McKim Formation.

Most of the geochemical data reported in this study are from the Gowganda Formation. They have been separated on Fig. 4 according to whether they represent diamictite matrix material or laminated argillites. All the data from Gowganda diamictites have been grouped together on a single histogram and plotted below the argillites but these two rock types are in fact interbedded in the formation².

Results from the three areas of Gowganda outcrop (Fig. 1) are differentiated on the histogram of CIA values shown on Fig. 4 but are not significantly different. The average value for the diamictite matrix material (56) is the lowest obtained from Huronian mudstones and supports the glacial interpretation of the Gowganda Formation. CIA values from the Gowganda

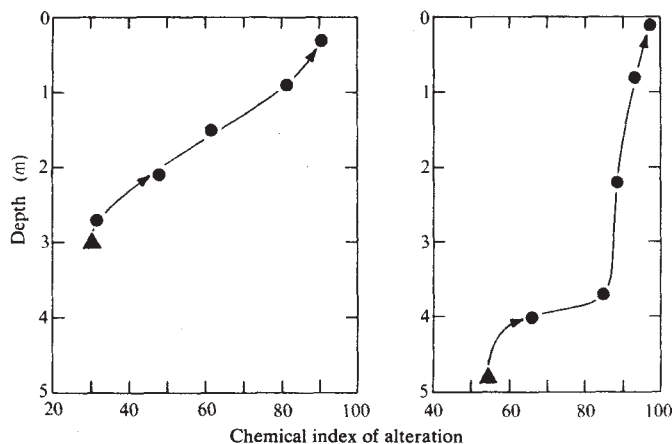


Fig. 3 Relationship between depth and chemical index of alteration in Recent weathering profiles developed on a basalt¹⁵ (a) and a granite¹⁶ (b) in Guyana. In each case the triangle represents fresh rock with successively higher CIA values upward in the weathering profile.

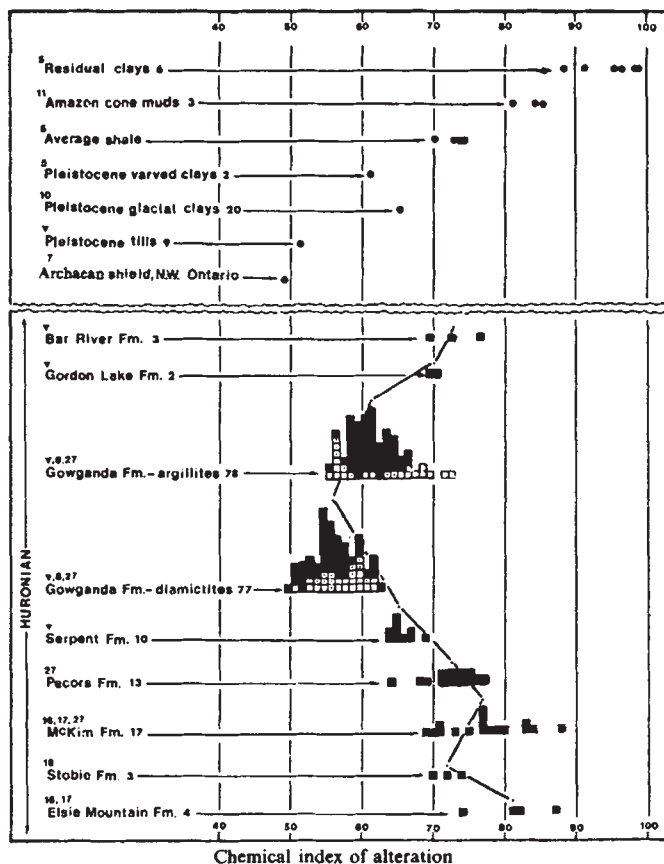


Fig. 4 Values of chemical index of alteration (CIA) for Huronian formations and various other rocks and sediments (upper part of diagram). Each square in the lower part of the diagram represents a single chemical analysis. Black dots in the upper part of the diagram are either individual analyses or averages. Numbers following formation names and so on indicate the number of chemical analyses involved. Numbers above formation names at left side are sources of data obtained for this study. ▲ (at left) indicate new data. For the Gowganda Formation □ are data from the Cobalt area (area 3); ◻ are from the Bruce Mines area (area 1); ■ are from the Whitefish Falls area (area 2).

diamictites are comparable with those derived from Pleistocene tills on the Archaean shield (Matachewan tills of Fig. 4).

Argillites of the Gowganda Formation yield CIA values higher than those of associated diamictites, reflecting incorporation of slightly more weathered materials. These argillites, however, contain less weathered materials than the average shale (Fig. 4) due to incorporation of glacial flour. Some samples from the northeastern part of the Huronian outcrop belt (area 3, Fig. 1) have relatively high CIA values, suggesting incorporation of more highly weathered material.

In general CIA values from the Gowganda argillites indicate that they were derived from muds of unusual composition, containing a relatively low proportion of alumina. Pleistocene varved and other glacial clays give similar low CIA values (Fig. 4) supporting the glacial origin of the Gowganda argillites and suggest that these form a chemically distinctive category of relatively unweathered muds and mudstones.

Data from lutites in Huronian formations above the Gowganda are few. Samples from the Gordon Lake and Bar River Formations (Fig. 4) have values similar to those of average shales and support the idea of climatic amelioration^{9,12} following deposition of the Gowganda Formation.

The geochemical evidence presented above suggests that the lowest Huronian formations, and in particular the Elsie Mountain and McKim Formations, contain significant amounts of highly weathered detritus. It is not known whether this material was derived by erosion of older palaeosols or by weathering contemporaneous with deposition. In the former case remark-

ably thick palaeosol development would be indicated, for the McKim Formation is up to 2,000 m thick and is extensive. In either case such a high degree of chemical alteration is suggestive of weathering in humid, possibly tropical conditions. Geochemical results from the Gowganda Formation confirm the widely held belief that it was deposited in a glacial environment. High CIA values for the overlying formations suggest an amelioration of climate.

The general interpretation based on CIA values suggests deposition in low latitude, possibly tropical conditions followed by climatic deterioration and subsequent amelioration. If the changes recorded in the Huronian Supergroup were related simply to global climatic variations then similar changes should be recorded in many other early Proterozoic sequences. Glacial deposits of comparable age to the Huronian are, however, relatively scarce⁹. An alternative interpretation of these results, based on uniformitarian principles, is that the Huronian succession records migration of the depositional site through various climatic zones. In present conditions, climatic variations as dramatic as those proposed for the Huronian succession would require a minimum change in latitude of about 30°. As the Huronian succession is thought to record a warm humid climate with subsequent deterioration and amelioration, migration of the depositional basin through a total distance equivalent to about 60° latitude is required on a uniformitarian basis. Current rates of seafloor spreading are typically in the range from 2 to 8 cm yr⁻¹ (ref. 25). These rates of spreading would require 350 and 100 Myr respectively to allow a 60° latitudinal spread. These estimates are calculated on the assumption that spreading took place in a direction normal to the palaeoequator and are therefore minimum values. They fall within the limitations suggested by available radiometric dates for deposition of the Huronian Supergroup (~2,500–2,100 Myr). The above estimates of variation in palaeolatitude during Huronian deposition are also in agreement with recent palaeomagnetic studies²⁶ suggesting that volcanic rocks in the lower part of the Huronian were extruded at a palaeolatitude of about 32° and that the Gowganda Formation was deposited at about 60° of latitude. Recent studies of early Proterozoic successions in the northern Great Lakes region^{27,28} and elsewhere in the Canadian Shield²⁹ support the existence of plate tectonics at that time. Although the solutions presented above is not unique, a uniformitarian approach, involving plate tectonic processes and latitude-related climatic variations, can be used to interpret the ancient rocks of the Huronian Supergroup.

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^{10}Be in island-arc volcanoes and implications for subduction

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We have measured the concentration of the cosmogenic isotope ^{10}Be in the lava of 19 separate flows, 15 of which are from island-arc volcanoes. We report here that 13 of the island-arc samples show concentrations between 2.7 and 6.9×10^6 atoms per g. Three non-island-arc and two island-arc samples have concentrations of 0.3×10^6 atoms per g or less, values only slightly higher than the processing blanks. Both island-arc samples having low concentrations are from rear rank volcanoes. The results of the low concentration samples indicate that the production of ^{10}Be by natural radioactivity or surface contamination is probably not important, although one sample of a Columbia Plateau flood basalt has a concentration of 1.0×10^6 atoms per g. We suggest that the higher concentrations in the island-arc samples result from the ejection of material that was once on the ocean floor, where a thin but concentrated layer of ^{10}Be resides, and that it was carried down with the subducted lithospheric plate.

The distinctive characteristics of the volcanoes ringing the great Pacific Basin and lining the Java-Sumatra arc were recognized long before the current ideas that relate them to subducting lithospheric plates found favour. Nevertheless, it was the notion of a dynamic mantle that gave the study of this large group a new importance. Their location near deep trenches and their regular spacing strongly suggested a common source, in some way related to the boundary defined by the Benioff zone, which is generally thought to delineate the upper surface of the down-going plate. The origin of the magma of these volcanoes remains controversial, with regard to both its place of formation and the sources of material on which it draws, a situation resulting from the difficulties encountered in interpreting the numerous chemical and isotopic data that are available. In the hope of introducing a new and possibly more direct observation into the question, we have measured the concentration of ^{10}Be in several basalts taken from island-arc volcanoes and other sources. A layer of pelagic ooze carried down by the subducting plate has a high concentration of the isotope, which has accumulated there after its descent from the top of the atmosphere where it is produced by cosmic rays primarily by spallation reactions on N and O. If the isotope is detected in island-arc magmas at concentrations much greater than in other basalts, and if it has not been introduced through contamination at the surface, carried into magma chambers by ground water or generated *in situ* by radioactivity, then one is compelled to attribute its presence as evidence for the emergence of subducted material that was once on the ocean floor. The 1.5×10^6 yr half life of ^{10}Be is perfect for this study because it is long enough to function as a tracer for the tectonic activity, yet short enough to disappear from recycling on a longer time scale.

Although it is more than 25 yr since ^{10}Be was found in deep ocean sediments¹, because of its long half life, the techniques of radioactive counting prevented its detection at concentrations much below $\sim 10^9$ atoms per g, and even at these high levels the technique was difficult. The introduction of accelerator mass spectrometry^{2,3} now enables us to measure

down to the concentrations reasonably expected in island-arc basalts.

We have used the method with a tandem Van de Graaff accelerator and applied it earlier to easier measurements^{4,5}. To obtain the data reported here it was necessary to attain the following operating characteristics: (1) a beam of $^9\text{BeO}^-$ of at least $1 \mu\text{A}$ for 2 h from a charge of 0.5 mg of Be in the form of BeO, (2) an overall accelerator transmission greater than 10% and (3) a $^{10}\text{B}^{3+}$ beam sufficiently intense to steer the $^{10}\text{Be}^{3+}$ beam but not exceeding 10^9 particles s^{-1} . This upper limit ensures that the ^7Be produced by the reaction $\text{H}(^{10}\text{B}, \alpha)^7\text{Be}$ when the ^{10}B beam strikes the hydrogen adsorbed in the detector foils does not limit the measurement sensitivity. Typical $E - \Delta E$ spectra for a high and a low level sample are shown in Fig. 1.

The concentration of ^{10}Be is measured by isotope dilution. A ^9Be spike of ~ 1 mg is added to ~ 10 g of rock during its dissolution in HF. The extraction chemistry is based on the stability of BeF_3^- and its bonding with anion exchange resin and is described elsewhere⁶. For these measurements it was necessary to scale up the procedure to accommodate 10 g samples. The amount of ^{10}Be present is calculated from the measured $^{10}\text{Be}/^9\text{Be}$ ratio, neglecting any natural ^9Be that may be present, a procedure that introduces an error that is small compared with the error in measuring the $^{10}\text{Be}/^9\text{Be}$ ratio, assuming that the sample contains Be at a level less than 10 p.p.m. The processing blanks are 1 mg quantities of ^9Be that are subjected to all phases of the extraction chemistry except the pulverization of the rock in a tungsten carbide crusher. Table 1 gives results of our measurements on 19 separate lavas.

It was originally feared that contamination of the lava by rain would be a problem, but this does not now seem likely. We know that rain has typically 1.5×10^7 atoms per l (refs 5, 6), which means that about 500 volumes of rainwater would have to penetrate the rock to produce the levels observed in several of the island-arc lavas. Furthermore, as soils quickly remove Be from rain, in practice orders of magnitude more ground water would be required. (We have measured a sample of well water from Maryland and found the ^{10}Be concentration to be not more than 10^5 atoms per l.) To minimize the risk of water contamination all samples were cut from the centres of relatively large pieces of lava and were leached with 0.5 M HCl in an ultrasonic cleaner before pulverization. Another possible source of contamination could result from the mixing of the magma with surface soils at the time of eruption. The concentration of ^{10}Be grows with the age of a soil, at the rate of about 3,000 atoms per g per yr (ref. 7). It seems unlikely that undisturbed weathering would occur in the region around volcanoes over the long period needed to form soil concentrations high enough for small admixtures to be a large source of error. Other sources of crustal contamination can be neglected. The consistently high concentrations in the 13 Central American and Aleutian samples, which average 4.5×10^6 atoms per g, and the low concentrations in the non-island-arc samples, which average 0.5×10^6 atoms per g, suggest that the former group has a source of ^{10}Be not shared with the latter. The low ^{10}Be content in the Japan and Taiwan samples, 0.1×10^6 and 0.3×10^6 atoms per g respectively, may result from their being rear rank volcanoes, the front rank having drawn off most of the surface material where the ^{10}Be resides. The 1.0×10^6 atoms per g concentration of the 14-Myr-old Columbia Plateau flood basalt, although much lower than that of the Central American and Aleutian samples, certainly gives one pause. It may be that we are observing in the Columbia Plateau basalt and in St George flow SG-11 a component of the ^{10}Be produced by radioactivity through the reaction $^7\text{Li}(\alpha, p)^{10}\text{Be}$. Li, U and Th tend to reside in different mineral phases and the effective range of the α -particles for initiating this reaction exceeds $10 \mu\text{m}$ for only a few of the α -emitters. This may explain the high value for the Columbia Plateau basalt, because it is extremely fine grained and has a substantial amount of glass. Another explanation may be the production of ^{10}Be directly by cosmic rays as

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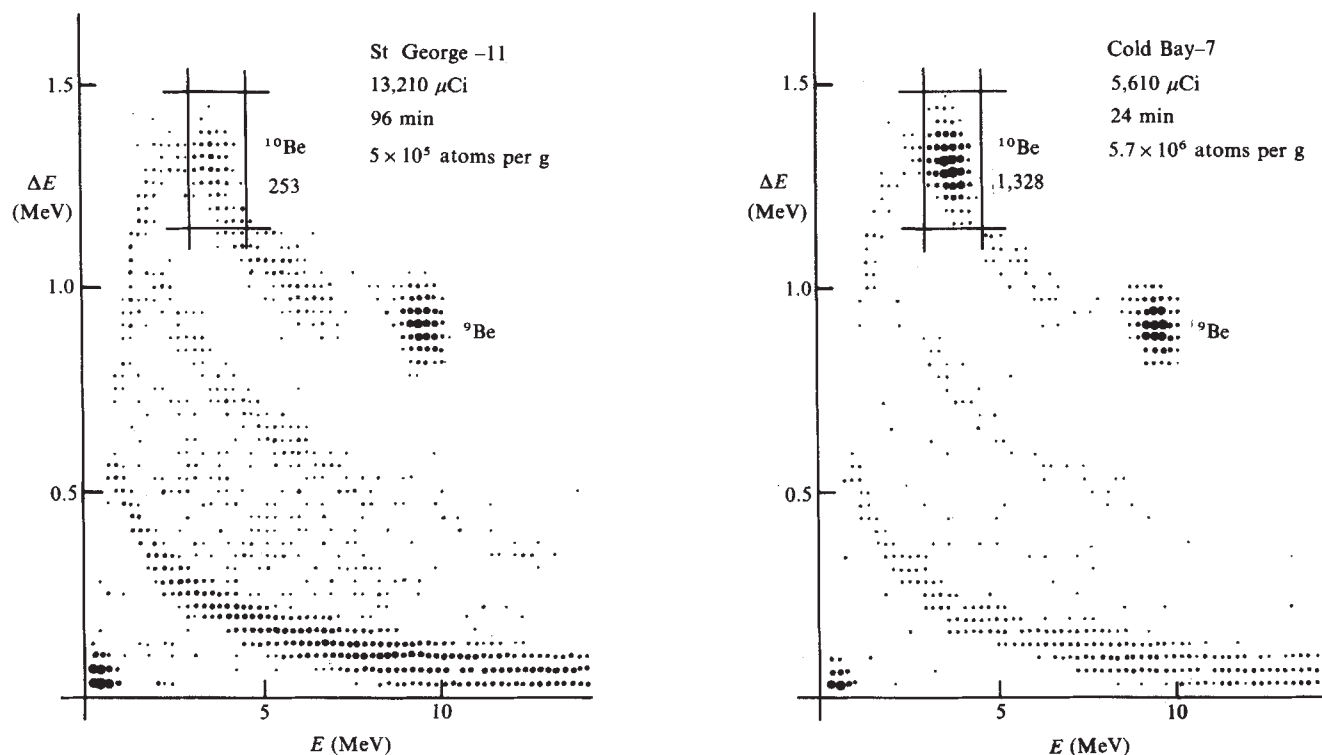


Fig. 1 Two-dimensional $E-\Delta E$ spectra obtained for two samples. After passing through an absorber that completely stops the ^{10}B and reduces the ^{10}Be energy from 23.7 MeV to about 4 MeV, the incident particles pass through a thin gas ionization counter and are finally stopped in a surface barrier detector. The former produces a signal, ΔE , related to atomic number, the latter a signal, E , proportional to the remaining energy of the particle. The radius of the dots is proportional to the logarithm of the number of counts in a given bin, with the smallest dots designating a single count. The rectangle designates the area integrated for ^{10}Be . Background extends through the rectangle and down to the right and is the ^9Be produced by the reaction $\text{H}(^{10}\text{B}, \alpha)^7\text{Be}$ from hydrogen in the absorber foil. The ^9Be peak results from the injection of $^9\text{BeOH}^-$ followed by charge exchange processes in the accelerator and beam transport system. $^9\text{BeO}^-$ is integrated continuously during the injection of $^{10}\text{BeO}^-$ into the accelerator. Results are given without background subtraction (for details see ref. 4).

a result of this rock having lain near the surface for much of its existence. At the 2,000-m altitude where it was found the production rate is 9 atoms per g per yr (ref. 8) and the equilibrium concentration is 2×10^7 atoms per g, far more than is observed. This calculation assumes that the sample lay at the surface for millions of years; however, a cover of less than

500 g cm^{-2} would have reduced the concentration to the observed 1×10^6 atoms per g.

Despite the reservations just stated about the source of the ^{10}Be in the island-arc basalts, it is useful to see what such concentrations imply, if they resulted exclusively from the subducted material. The surface density of the isotope in the ooze

Table 1 Measurements of ^{10}Be concentration in various lavas

Volcano	Sample designation	Source	Rock class	^{10}Be (10^6 atoms per g)
Island arc volcanoes				
Cerro negro, Nicaragua	CN-I	A. Rubin	Basalt	6.8
Cerro negro, Nicaragua	CN-II	A. Rubin	Basalt	6.1
St. Maria, (Santiaguito Dome) Guatemala*	Ash	A. Rubin	Ash	3.3
St. Maria, (Santiaguito Dome) Guatemala*	Rock	A. Rubin	Dacite	2.9
Atka, Aleutian Islands	AT-4	B. D. Marsh	Basalt	4.2
Atka, Aleutian Islands	AT-87	B. D. Marsh	Basalt	3.6
Atka, Aleutian Islands	AT-112	B. D. Marsh	Basalt	3.4
Atka, Aleutian Islands	AT-129	B. D. Marsh	Basalt	2.7
Atka, Aleutian Islands	AT-130	B. D. Marsh	Basalt	4.6
Cold Bay, Aleutian Islands	CB-3	B. D. Marsh	Andesite	6.9
Cold Bay, Aleutian Islands	CB-7	B. D. Marsh	Andesite	5.7
Cold Bay, Aleutian Islands	CB-8	B. D. Marsh	Andesite	3.2
Cold Bay, Aleutian Islands	CB-13	B. D. Marsh	Andesite	4.5
Usu, Japan*		H. Okada	Andesite	~0.1
Ta-Tung, Taiwan	E-201	W. J. Cheng	Andesite	0.3
Non-island-arc volcanoes and flood basalts				
St. George, Pribilof Islands	SG-7	B. D. Marsh	Basalt	~0.1
St. George, Pribilof Islands	SG-11	B. D. Marsh	Basalt	0.3
Kilauea, Hawaii	HHP-66-1	J. Philpotts	Tholeiite	~0.1
Columbia Plateau flood	ORGR N ₂ -3	R. Carlson	Basalt	1.0
Processing blanks	LB-157			~0.1
	LB-179			~0.1

* Designates a sample obtained immediately after eruption. All samples are from flows that are recent compared with the lifetime of ^{10}Be . Analytical accuracy for data with concentrations $> 10^6$ atoms per g is 15%. For others it is of the order of 10^5 atoms per g.

when it reaches the trench is q/λ , where q is the precipitation rate and λ the decay rate; this assumes that radioactive decay and precipitation are in equilibrium. If this two-dimensional distribution descends as a sheet along or near the plate boundary and travels with a plate velocity v along a trajectory of length l , then the surface density at the roots of the volcanoes will be $q\lambda^{-1}\exp(-\lambda l/v)$. The maximum rate at which atoms of the isotope could be furnished to the roots of the chain over a front width W is $qvW\lambda^{-1}\exp(-\lambda l/v)$. If the average rate of mass ejected by the chain is $\rho\dot{V}$, then the maximum possible concentration of atoms from this source is

$$\eta = \frac{qvW}{\lambda\rho\dot{V}} \exp(-\lambda l/v)$$

Considering the Aleutians, $W = 2.1 \times 10^8$ cm and $\dot{V}$ lies between 7 and 30×10^{11} cm³ yr⁻¹ (ref. 9). The values of v and l are tabulated: 7.26 cm yr⁻¹ and 2.22×10^7 cm for Atka and 6.72 cm yr⁻¹ and 2.18×10^7 cm for Cold Bay¹⁰. The precipitation constant q probably lies between 1 and 3×10^6 atoms cm⁻² yr⁻¹. Taking intermediate values for the poorly known quantities gives maximum possible concentrations of 2.2×10^8 atoms per g for Atka and 1.8×10^8 atoms per g for Cold Bay. The average measurements for these two volcanoes are 1.7 and 2.8%, respectively, of the calculated values, the latter quantities having large uncertainties, probably a factor of 2. Data are not available for $\dot{V}$ for the Central American volcanoes, but the similarity of the results indicates the same magnitude of values.

Although recent chemical and isotopic studies suggest the inclusion of sedimentary material in the lavas of the Aleutian arc volcanoes¹¹⁻¹⁴, the present results provide by far the most direct evidence to support this supposition. Unless radioactivity or surface contamination prove to be much more important sources than they now appear, the presence of ¹⁰Be requires the inclusion of material that was on the surface within the past few million years. The existence of ¹⁰Be in island-arc lavas requires the transport of surface material and cannot result from contamination of the magma on its way to the surface by continental material. The conclusions of the present study are independent of assumptions regarding the composition or uniformity of composition of the mantle, asthenosphere, crust or pelagic ooze. The scatter of the Aleutian and Central American samples seems to us surprisingly small. It will be of interest to learn whether this consistency holds for a wider variety of front rank volcanoes and whether those in the rear rank always lose their ¹⁰Be.

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Depositional features of March 1982 Mount St Helens sediment flows

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On 19 March 1982, 2 yr after initial eruption, Mount St Helens erupted explosively, resulting in the deposition of new sediment flows in the Toutle River drainages (Fig. 1). We conducted field observations of these flows during the period 29 March–5 April 1982. By this time, the rivers, rapidly re-establishing pre-eruption channel levels, had down-cut through the new flows, allowing us to examine the internal structure of the flows before they could be extensively modified by reconstruction projects. The flows were characterized by a distinct three unit sequence. A basal massive to graded layer (unit 1) consists of large clasts in grain to grain contact. It is overlain by a distinctly finer-grained stratified unit (unit 2) of similar thickness. The top unit (unit 3) contains very large matrix-supported clasts and transported log debris (Fig. 2). This sequence has not previously been documented where its origin could clearly be ascribed to a single catastrophic depositional event. The sequence provides a useful model for interpreting similar deposits in the rock record.

Unit 1 is fairly well sorted and in many cases fines upward. There is a mixture of well-rounded and angular clasts, and although proportions vary depending on local sources, rounded stream-derived clasts usually dominate. We observed only one instance of strong preferred orientation of clasts. In this case many of the clasts are tabular, angular fragments apparently derived from the breached debris dam east of Camp Baker. These clasts are strongly oriented parallel to bedding.

In other deposits, sphericity of the clasts is high and would be unlikely to display a preferred direction. Clasts in unit 1 are in grain to grain contact with 10–15% matrix. Angular grains within this layer form a tightly interlocking texture which results in high resistance to erosion. In many deposits this unit already had the consistency of setting concrete only 13 days after deposition. Clast size is variable but related to overall unit thickness. The maximum clast size we observed within unit 1 is 40 cm, and the average size in the proximal sequences is estimated to be ~10–15 cm. In the distal sequences, clast size is 1–2 cm. The clasts are a mixture of locally derived red and grey intermediate volcanics, some vesicular basalt, and minor amounts of pumice. The average grain size of the matrix varies from sand to pebbles in proximal flows to fine silt in distal flows.

The contact between unit 1 and the overlying fine unit is sharp in most cases, although we observed some gradational transitions. Generally units 1 and 2 are roughly equal in thickness. Like the lower unit, the upper deposits are moderately well-sorted and in some cases exhibit normal grading. The contrast in grain size with unit 1 is marked; in the coarsest packages this part of the sequence has an average grain size of 3–5 mm. Crude horizontal laminae are ubiquitous. In some locations very distinct horizontal laminae 3–5 mm in thickness are developed. The water content of this part of the sediment flow is very high compared with the lower unit; thixotropic behaviour was observed in portions of unit 2 through the length

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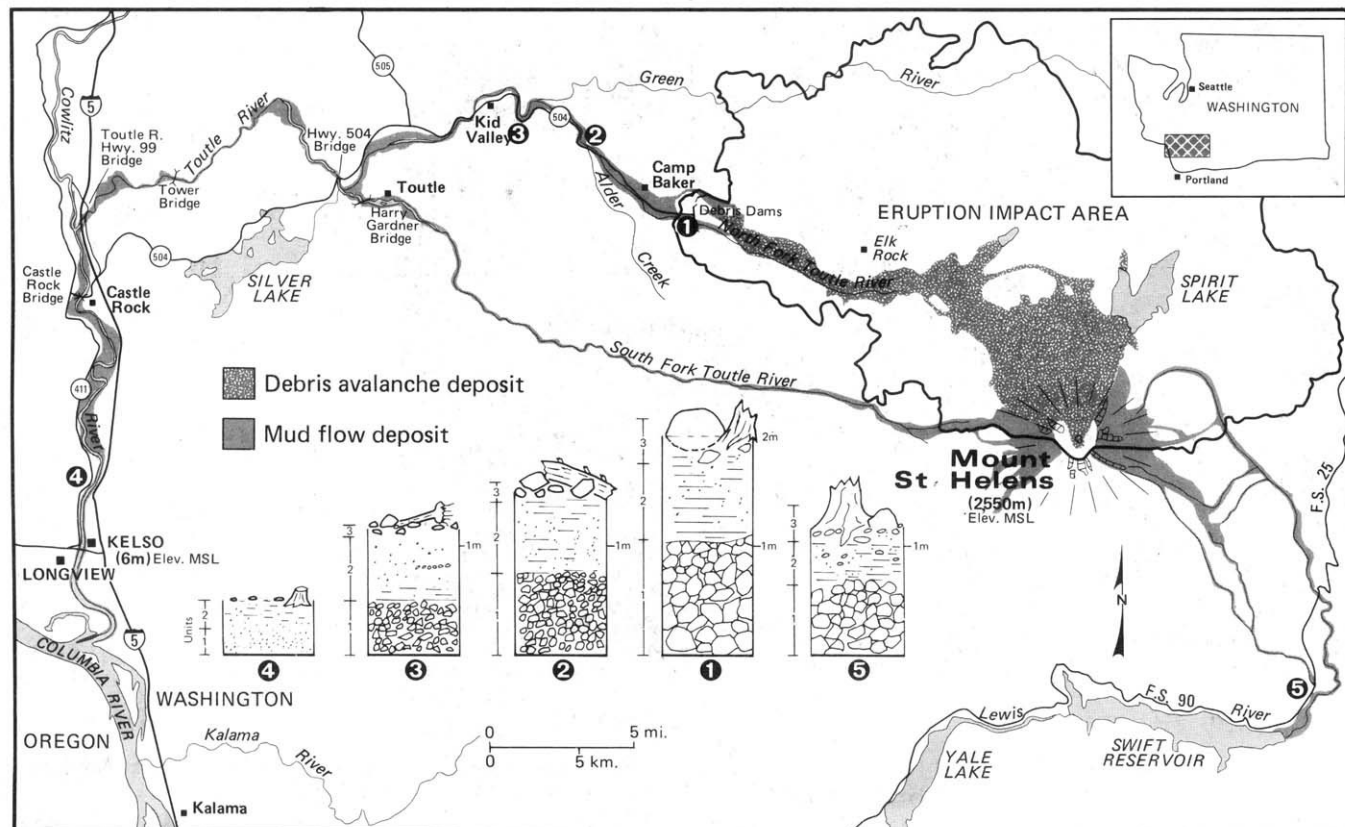


Fig. 1 Index map of the Mount St Helens area. Diagrammatic columns illustrate facies changes in the characteristic sequence of sediment flows. Clasts are drawn approximately to scale. Numbers 1–4 represent the 19 March 1982 sediment flow while no. 5 represents deposition during the sediment flows of 1980 only.

of the Toutle River sediment flows. In spite of the high water content, we observed no soft sediment deformation, possibly because of the strength of the underlying coarse layer. No lenses of coarse clasts appear in this unit, with the exception of rare stringers of rounded pumice fragments.

The uppermost layer and surface of the sediment flows is commonly strewn with very large matrix supported clasts up to 2 m in diameter and transported log debris. The average size of the clasts consistently exceeds that of the unit 1 clasts, so that in locations where unit 3 is present, the largest fragments in the sediment flow occur not in the basal unit but as matrix-supported clasts at the top of the sequence. The logs and stumps at this horizon are deposited in both horizontal and vertical orientations. Those which rest vertically are generally stumps with large root systems. Similar deposits of log debris were observed on the 1980 sediment flows^{1,2}. A layer of pumice fragments appears on the surface of the sediment flows, concentrated particularly near the margins of the flows or where trapped by other surface debris. These fragments are rather well sorted and fine markedly downstream. Roundness is high, even in the most proximal flows we observed.

The thickest sediment flows we were able to examine occurred at the sediment dam 2 km east of Camp Baker on the North Fork of the Toutle River (Fig. 1). Here the flows were up to 3.5 m thick, totally filling the sediment reservoir and actually extending above the dam surface. Most of the dam had filled before the March eruption; the March flows added an additional average thickness of sediment estimated at ~3 m. At the time we examined the flows, the river had breached the dam, and excavated a channel through the sediment to a depth of 3 m. Upstream from the sediment dam, the basal unit of the sequence is not well developed, probably because energy conditions and available sediment type changed abruptly once the dam was breached. Immediately downstream from the dam maximum thickness of the flows is ~2.5 m, and each of the three units is apparent. We were able to document this sequence

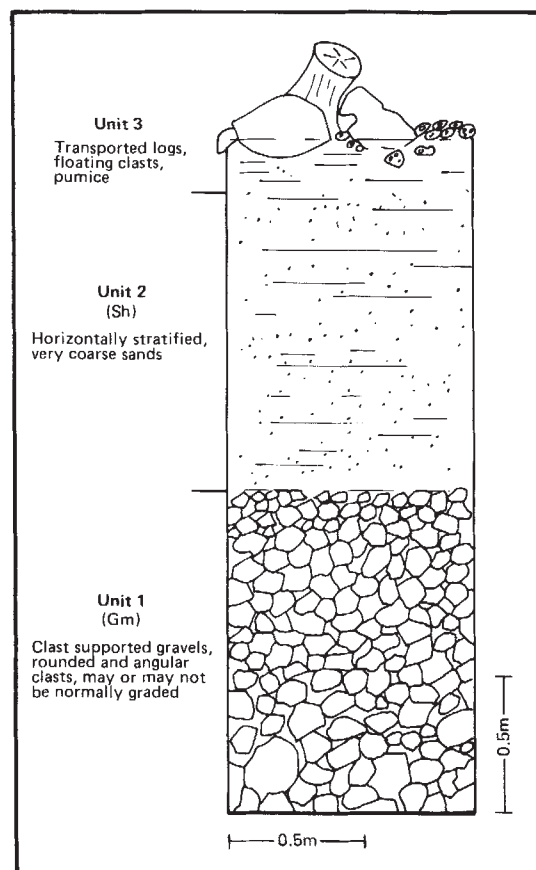


Fig. 2 Three-unit sequence of sediment flows based on proximal flows immediately downstream from debris dam.



Fig. 3 Photograph of the three-unit sequence 0.5 km downstream from debris dam. Shovel, 80 cm long.

downstream along the Toutle River and found it particularly well defined at the washout of Highway 504 east of Kid Valley and near the mouth of Alder Creek (Fig. 1). On the Lewis River at the Eagle Cliff Bridge (location 5, Fig. 1), a 2-m thick section of 1980 sediment flow displays the same sequence as noted in 1980 and 1982 flows on the Toutle. The size of the package decreases downstream as does the size of clasts within the sediment flows. Deposits on the Cowlitz River also form similar packages ~0.5-m thick; here the grain size varies from coarse sand at the base to very fine sand and silt at the top.

The processes that form the three unit sequence clearly require further study. We have avoided term 'mudflow', although its use is firmly established in both popular and geological literature³ about the Mount St Helens flows, because it connotes a sedimentological process probably inapplicable to these deposits. These flows actually contain little mud and lack the features of classic sediment flows of poor sorting, massiveness and dominance of matrix support⁴. Although the sorting and stratification of the lower units suggest fluid conditions, characterizing the flows as 'fluvial' would also be misleading. The density, sediment content and depositional rates of these flows are not within the scope of that term as it is usually used. Within a few hours of the eruption, the flows buried roads and equipment in the Toutle drainage and added an estimated 3 m to the surface of the sediment dam. Videotape taken by local television stations show large boulders and log debris floating at the surface of the flows. As this debris was carried and deposited at the top of the flow (unit 3) it not only demonstrates the density of the flows⁵, but clearly shows that the sequence formed during a continuous event. The presence of this 'mudflow-like' unit 3 at the top of the sequence also implies that fluvial reworking of the sediments subsequent to deposition has been minor.

The catastrophic origin of deposits in the rock record similar to the Mount St Helens flows could easily be overlooked, particularly if large angular clasts or floating debris were absent. Deposits ascribed to a fluvial origin contain sequences resembling units 1 and 2. Transitions from massive gravel to horizontally laminated sand, Gm to Sh lithofacies (Miall lithofacies code⁶), which characterize the Mount St Helens flows, are present in proximal braided streams as modelled by Miall's Scott profile⁶ and Rust's facies assemblage G_{II} (ref. 7). Steel and Aasheim⁸ describe couplets of massive conglomerate and

parallel laminated sandstone from braided stream deposits in the Devonian Hornelen Basin of Norway.

Several criteria may help distinguish ancient fluvial deposits from Mount St Helens type flows. Trough cross-beds and ripple laminae are common though not essential features of proximal braided river deposits⁶, but are almost entirely absent from the sediment flows. The presence of unit 3 type deposits would certainly suggest a sediment flow origin, and volcanic composition and angularity of clasts could corroborate this interpretation. Further study of the mechanics of deposition of the Mount St Helens type flows may reveal other distinguishing criteria.

Correctly identifying deposits in the rock record with an origin like that of the Mount St Helens flow will significantly affect interpretation of their geological setting. Whereas the features we have documented resemble certain fluvial deposits, they clearly formed during extremely rapid deposition resulting from catastrophic sediment flows. Within only 2 yr, the two major Mount St Helens sediment flow events have each resulted in deposits ranging from 1 to 4 m thick³. The repetition of these events suggests another important conclusion: the deposits are likely to be cyclical. Similar sequences were observed in the 1980 Mount St Helens flows, and we were able to discern the sequence in remnants of the 1980 flows which we examined this spring. We have also noted strikingly similar cyclic deposits in Tertiary volcanoclastic sediments in the Western United States⁹. We believe the Mount St Helens flows will provide a useful model for interpreting and reinterpreting volcanoclastic deposits in the rock record.

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Redividing the basidiomycetes on the basis of 5S rRNA sequences

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Sequence data from slowly evolving macromolecules have been recognized for at least two decades¹ to be of value in reconstructing the ordering and timing of evolutionary events as far back as the origins of eukaryotes and prokaryotes^{2,3}. Fungi are particularly suitable organisms for investigating phylogenetic relationships by this approach. The fossil record is poor⁴ and morphological characters are limited, providing few reliable markers of phylogenetic relationships. To date, nearly all molecular sequence data from fungi have been derived from ascomycetes^{5,6}, primarily yeasts. Basidiomycetes are represented by only one relevant macromolecular sequence, that of the cytochrome *c* from *Ustilago sphaerogena*⁷. Here, we report 5S ribosomal RNA (rRNA) nucleotide sequences from eight species of basidiomycetes. These sequences define two distinct clusters that correlate with the presence or absence of cell wall septal dolipores rather than with the traditional division of these species between the classes^{8,9} Heterobasidiomycetae and Homobasidiomycetae. We believe this provides a striking example of the utility of molecular sequence data in distinguishing reliable morphological or cytological marker(s) for a given phylogenetic lineage.

5S rRNA was isolated¹⁰ from French press lysates of the yeast phase of all heterobasidiomycetes and from the mycelia of the homobasidiomycetes, grown axenically in liquid cultures. The 5S rRNA was end-labelled with ³²P (ref. 11). 3'-Terminally labelled molecules were subjected to nucleotide-specific partial digestion¹² and 5'-terminally labelled molecules were partially hydrolysed by several ribonucleases with differing nucleotide specificities^{13,14}. Sequence ladders were obtained from these partial digests on polyacrylamide gels^{10,12}. Terminal nucleotide assignments were confirmed by TLC¹¹. The sequences (Fig. 1) are aligned to indicate an insertion in the first three in position 109. (With the limited sequence data, this insertion could also plausibly be ascribed to the uridine in position 107.) The order of nucleotides 4–6 in the sequences for *Ustilago violacea*, *Rhodospiridium toruloides* and *Aeossosporon salmonicolor* indicated by the 5'-terminally labelled partial enzyme digests rather than those indicated by the 3'-terminally labelled partial chemical digests was assumed to be correct. The former ordering was more consistent, and provides a better fit with current models of secondary structure. These sequences generally fit the secondary structural model for eukaryotic 5S rRNA of Böhm *et al.*¹⁵ and Diels *et al.*¹⁶. Several mispairs in helical regions are evident; for example, U:U mispairs between positions 2 and 118 in sequences 4–8, and a novel looped-out nucleotide (the uridine in position 107) in helix D (helix V according to some authors^{17,18}) in sequences 1–3.

These sequences define two clearly distinct clusters (Table 1). Sequences within each cluster differ by no more than 13

Table 1 Matrix of pairwise comparisons of number of nucleotide positions with a different nucleotide

Species	Nucleotide differences							
	Adoliporous				Doliporous			
	U.v.	R.t.	A.s.	F.f.	F.c.	T.m.	S.c.	B.a.
U.v.	—	9	4	43	44	42	42	44
R.t.	9	—	6	40	39	39	39	39
A.s.	4	6	—	43	43	45	42	44
F.f.	43	40	43	—	3	13	5	2
F.c.	44	39	43	3	—	10	4	2
T.m.	42	39	45	13	10	—	8	10
S.c.	42	39	42	5	4	8	—	4
B.a.	44	39	44	3	2	10	4	—

The species are given by their initials in the same order as given in Fig. 1, and are segregated according to whether or not they have septal dolipores.

nucleotides while sequence differences between the two clusters average 42 nucleotides. (This compares with a difference of eight nucleotides between the human and trout sequences, for example⁶.) Surprisingly, these clusters do not correspond to the generally accepted affinities of these species with the classes⁹ Heterobasidiomycetae and Homobasidiomycetae^{19,20} (Table 2). The sequences from the two yeast-like species of *Filobasidium* (heterobasidiomycetes) differ by only a few nucleotides from those of the two homobasidiomycetes *Schizophyllum commune* (a stalkless gill fungus) and *Bjerkandera adusta* (a shelf fungus). The sequence from the jelly fungus *Tremella mesenterica* is the most divergent within that cluster which includes species from both classes of basidiomycetes. This suggests that the deviations from typical homobasidiomycete morphological characteristics in *Tremella* and *Filobasidium* are probably of independent origins.

Although the 5S rRNA genes of basidiomycetes have not been examined, typically there are many such genes in eukaryotes, either in tandem²¹ or dispersed through the genome²². Thus, different minor variants could undergo substitutions over long time periods before becoming the major expressed variant in closely related species, resulting in the erroneous impression that these species are only distantly related. Also, the major variant expressed in given tissues or developmental stages of an organism may differ^{23,24}. These present potential limitations on the confidence in phylogenetic conclusions based solely on rRNA sequence data. In the present case, since RNA was extracted from the haploid yeast phase of all heterobasidiomycetes, it is unlikely that the two clusters of sequences from the heterobasidiomycetes represent products of distinct genes expressed at different stages in the life cycle. Furthermore, given the large number of nucleotide differences between the two clusters of sequences, the probability that the cluster including both heterobasidiomycetes and homobasidiomycetes represents convergence of a variant gene lineage in these heterobasidiomycetes with the homobasidiomycete sequences is rendered minute.

Two basic types of cell wall septal pores have been observed among basidiomycetes: the morphologically complex dolipore and 'simple' pores²⁵. The latter are usually regarded as resembling the septal pores of ascomycetes^{25,26}, though lacking the

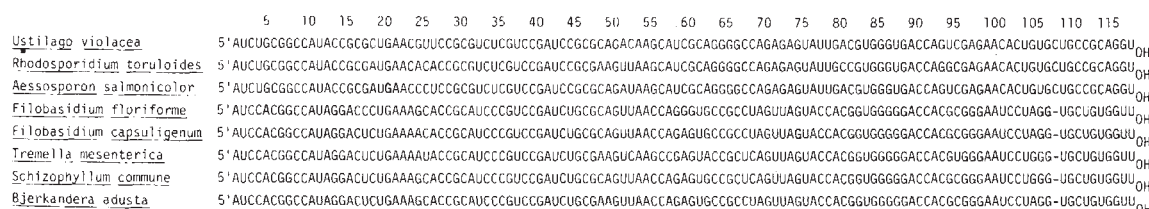


Fig. 1 The nucleotide sequence of the 5S rRNAs of eight species of basidiomycetes. Numbering of positions begins at the 5' end. Original data have been supplied to referees and are available on request from W.F.W.

Table 2 Comparison of the species of basidiomycetes

Species	Salient morphological descriptor	Class	Septal pore type
U.v.	Smut	Heterobasidiomycetae	None*
R.t.	Yeast-like	Heterobasidiomycetae	Simple
A.s.	Yeast-like	Heterobasidiomycetae	Simple*
F.f.	Yeast-like	Heterobasidiomycetae	Dolipore
F.c.	Yeast-like	Heterobasidiomycetae	Dolipore
T.m.	Jelly fungus	Heterobasidiomycetae	Dolipore
S.c.	Sessile gill fungus	Homobasidiomycetae	Dolipore
B.a.	Shelf fungus	Homobasidiomycetae	Dolipore*

* These species apparently have not been examined for dolipores; however, species or genera regarded as closely related have been examined.

Woronin body characteristic of the latter²⁶. Dolipores, although first described in detail only 20 years ago²⁷, apparently are universal, or nearly so, among homobasidiomycetes²⁵, except Exobasidiales²⁸, and are present in some imperfect species (that is, those lacking a known distinct sexual stage)²⁹⁻³². In contrast, many heterobasidiomycetes examined, including the rusts, ustilaginaceous smuts and most yeast-like species, have only 'simple' pores or no septal pores^{25,29,33}. Among those heterobasidiomycetes which do have dolipores are the jelly fungi genus *Tremella*^{34,35} and the yeast-like species of *Filobasidium*²⁹ (Table 2). In contrast, dolipores have not been found in *Rhodospidium*²⁹, *Ustilago*³³ nor in the yeast *Sporidiobolus*, generally believed to be closely related to *Aessosporon*³⁶. Thus, the presence or absence of dolipores in the species from which sequence data were obtained corresponds precisely with the two clusters of sequences. It will be of interest to determine whether sequences from other groups of heterobasidiomycetes with septal dolipores^{27,33,37} also cluster with the sequences here determined from doliporous species.

The high degree of sequence divergence between 5S rRNAs from doliporous and adoliporous species is comparable to that between each group and sequences from several other phyla⁹ of fungi (ref. 38 and unpublished data), although somewhat less than the difference between each cluster and most ascomycete sequences⁶. Indeed, in our (unpublished) phylogenetic analysis of fungal 5S rRNA, we are unable to show clearly that these two groups of sequences are more recently diverged from each other than from sequences from chytrid water moulds. However, the approximate indicated time of divergence of the basidiomycete and ascomycete sequence (~700 Myr ago) is in approximate agreement with most prior analyses of cytochrome *c* sequence data³⁹, reflecting a much faster apparent rate of substitutions within the fungi than within the metazoa.

Thus, the 5S rRNA sequence data support the prevalent but controversial⁴⁰⁻⁴² concept of an early dichotomy, producing two distinctive lineages among present basidiomycetes. However, it appears that the presence or absence of septal dolipores may be a more reliable morphological expression of this dichotomy than the sometimes contradictory combinations of traits involving reproductive structures and spore germination⁸ that traditionally separate the homobasidiomycetes and heterobasidiomycetes. In addition, species lacking distinct sexual structures may be recognizable as belonging to one of these lineages, based on septal pore ultrastructure. All of the adoliporous species from which sequences were determined are included in the provisional phylum Ustomycota, proposed by Moore⁴³. Whether sequences from rusts and the several other groups^{28,44,45} with only 'simple' septal pores will fall within this cluster remains to be determined. Unfortunately, nearly all of these remaining adoliporous groups are plant or insect parasites or associates and are difficult or impossible to culture on artificial substrates at present.

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Reiteration of nitrogen fixation gene sequences in *Rhizobium phaseoli*

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Bacteria of the genus *Rhizobium* form symbiotic nitrogen-fixing relationships with legumes. To investigate the organization of the nitrogen-fixation (*nif*) genes in *Rhizobium phaseoli*, which nodulates *Phaseolus vulgaris*, we have taken advantage here of the sequence homology between the *nifD* and *nifH* genes of *Klebsiella pneumoniae* and those of other nitrogen-fixing species¹⁻³. Strains from different geographical origins were studied, and a common pattern of organization of *nif*-homologous sequences was found. Initial characterization of *nif* sequences in *R. phaseoli* has revealed the presence of stable reiterations of DNA sequences including *nif* sequences, and suggests that at least some of these sequences are located on a large plasmid.

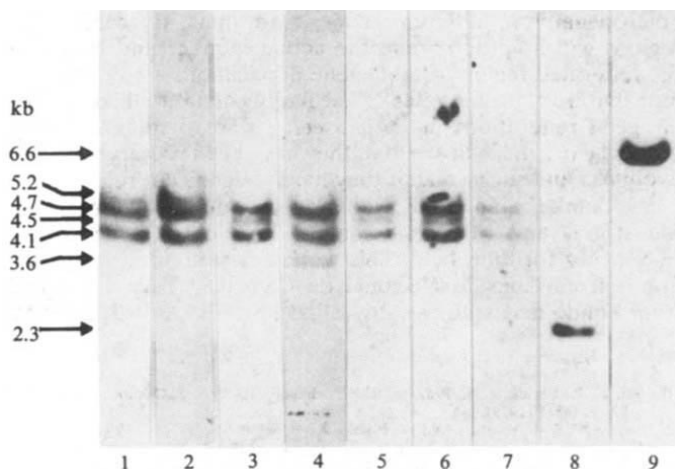


Fig. 1 Hybridization of *K. pneumoniae* *nif* genes with *Rhizobium* DNA sequences. Total DNA from different strains was digested with *Eco*RI restriction endonuclease. Digests (3 μ g for *Rhizobium* strains, and 0.5 μ g for *Klebsiella*) were separated on 0.8% agarose gels and transferred to nitrocellulose filters⁵. Blots were hybridized with the *Eco*RI insert of pSA30 plasmid labelled with ³²P to high specific activity (10⁸ c.p.m. per μ g DNA) by nick translation¹¹. For each lane 10⁶ c.p.m. of probe DNA was used. Hybridization was performed at 42 °C for 12 h in the presence of 50% formamide, 5 $\times$ SSC, 0.1% SDS, 1 mM EDTA, 10 mM Tris pH 7.6, 5 $\times$ Denhardt's solution²⁰ and 100 μ g ml⁻¹ of calf thymus DNA. Autoradiography in the presence of intensifying screens was carried out at -70 °C for 18 h. Lanes 1-4, *R. phaseoli* native isolates from México: 1, strain CFN-Rph1; 2, strain CFN-Rph5; 3, strain CFN-Rph42; 4, strain CFN-Rph215. Lanes 5-7, *R. phaseoli* from collections of other countries: 5, strain CIAT281 from Colombia; 6, strain Nitragin 17 from USA; 7, strain 8251 from the John Innes Institute collection, UK. Lane 8, *R. leguminosarum* strain T3 from the John Innes Institute collection, UK. Lane 9, *K. pneumoniae* wild type strain Kp5022. Arrows indicate length in kilobases of the different DNA restriction fragments and were calculated using λ phage DNA digested with *Hind*III as marker.

The strains used in this study were selected on the basis of their ability to infect and to fix nitrogen in *P. vulgaris*, as well as their capacity to produce a dark pigment (probably melanin) after prolonged incubation in culture media⁴, characteristics that have been associated with *R. phaseoli* but not with other cross-inoculation groups. These strains include native isolates from Mexico as well as strains derived from collections of other countries. Total DNA from different strains was digested with the restriction endonuclease *Eco*RI, separated by electrophoresis in agarose gels, and transferred to nitrocellulose membranes⁵. These blots were hybridized with the 6.6-kilobase (kb) *Eco*RI DNA fragment derived from the plasmid pSA30, which contains the structural genes for nitrogenase and nitrogenase reductase of *K. pneumoniae*⁶. Two hybridizing bands, with lengths of 4.7 kb (band *a*) and 4.1 kb (band *b*), are common to all of the strains screened (Fig. 1). A third band (*c*), with a less intense hybridization signal, is found in all strains, and has a size of 5.2, 4.5 or 3.6 kb, depending on the strain. In contrast, a *R. leguminosarum* strain included in this study yields only one hybridization band, with a size of 2.3 kb. The pattern we have found for *R. phaseoli* strains is consistent with earlier reports^{2,7}; however, different patterns were found for other species^{1,2,7,8}.

To analyse further the organization of *R. phaseoli* *nif* sequences, *Eco*RI fragments from strain CFN-Rph-42 were cloned in pBR328⁹. Transformed *Escherichia coli* colonies were screened¹⁰ for hybridization with the pSA30 *Eco*RI insert. Of 10,000 colonies screened, 27 positive clones, containing plasmids with single inserts, were obtained, including 10 with the 4.7-kb band (*a*), 13 with the 4.1-kb band (*b*), and 4 with the 4.5-kb band (*c*). The low recovery of clones containing band *c* could be related to the less intense hybridization signal associated with this DNA region. Recombinant plasmids containing

inserts corresponding to the *a* (pCQ15), *b* (pCQ12) and *c* (pCQ23) bands were isolated and the inserts purified by preparative agarose-gel electrophoresis. These inserts were labelled with ³²P by nick translation¹¹ and hybridized with Southern blots of total DNA digested with *Eco*RI from three different strains. Band *c* has a different size in each of these strains. As shown in Fig. 2, all three inserts *a*, *b* or *c* hybridize to all three bands that give positive signals with *K. pneumoniae* *nif* gene probes. This result indicates that the inserts contain DNA sequences that are reiterated in the genomes of the strains analysed. Inserts *a* and *b* from strain CFN-Rph42 give strong hybridization signals with bands *a* and *b* from each strain and a less intense signal with band *c*. Insert *c* gives a strong signal with band *c* of all three strains, in spite of the fact that this band has a different size in each of them.

To determine which *Klebsiella* *nif* genes are hybridizing with the *R. phaseoli* DNA sequences, the pSA30 *Eco*RI insert was digested with *Hind*III, *Pst*I and *Sal*I restriction endonucleases and the fragments obtained were separated by electrophoresis, blotted onto nitrocellulose and hybridized with the *Eco*RI inserts from plasmids pCQ15 (*a*), pCQ12 (*b*) and pCQ23 (*c*). As shown in Fig. 3, the different *R. phaseoli* sequences hybridize with only a 3.6-kb *Hind*III, a 3.0-kb *Pst*I and a 3.3-kb *Sal*I fragment. The positions of the nitrogenase genes in the *Eco*RI fragment of *K. pneumoniae* DNA have been reported by different groups^{6,17,18}, and allows us to conclude that each of the three *R. phaseoli* clones contains sequences that hybridize to the *K. pneumoniae* *nif* *D* (a nitrogenase subunit) and *nif* *H* (nitrogenase reductase) region. This agrees with previous reports that in stringent conditions, hybridization of *Rhizobium* sequences with *Klebsiella* *nif* genes is confined to the *nif* *D* and *nif* *H* genes^{1,2,8,12}.

Several recent reports have suggested that at least some *Rhizobium* *nif* genes are located on large plasmids^{7,13-15}. We

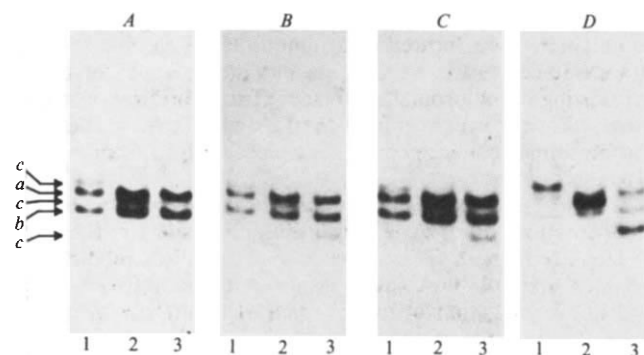


Fig. 2 Hybridization of cloned *R. phaseoli* *nif*-related DNA sequences with total DNA from different *R. phaseoli* strains. DNA fragments from strain CFN-Rph42 were inserted into the *Eco*RI site of plasmid pBR328⁹. Transformed *E. coli* colonies were screened for hybridization with the ³²P-labelled *Eco*RI insert derived from plasmid pSA30. Positive clones were further analysed by *Eco*RI digestions and hybridization with the pSA30 *Eco*RI insert. Plasmids containing inserts representative of each of the three fragments that hybridize with *Klebsiella* *nif* genes: pCQ15 (4.7 kb, band *a*), pCQ12 (4.1 kb, band *b*) and pCQ23 (4.5 kb, band *c*) were isolated. *Eco*RI inserts from each plasmid were purified and labelled with ³²P at high specific activity (10⁸ c.p.m. per μ g DNA) by nick translation¹¹. These labelled inserts, as well as the pSA30 *Eco*RI insert were used as probes for hybridization with Southern blots of *Eco*RI digests of DNA from different *R. phaseoli* strains. A, hybridization performed in 50% formamide as described in Fig. 1; pSA30 *Eco*RI insert as probe. B-D, hybridization performed at 65 °C for 12 h in the presence of 0.1 M sodium phosphate buffer pH 6.8, 5 $\times$ SSC, 10 $\times$ Denhardt's solution and 100 μ g ml⁻¹ calf thymus DNA; *Eco*RI inserts from pCQ15 (B), pCQ12 (C) and pCQ23 (D) were used as hybridization probes. For each lane 10⁶ c.p.m. of probe DNA was used and autoradiograms were from an 18-h exposure. Blotted DNAs were: strain CFN-Rph1 (lanes 1), strain CFN-Rph42 (lanes 2); strain CFN-Rph215 (lanes 3). Arrows indicate positions of *a* and *b* bands as well as alternative positions of band *c* (see text).

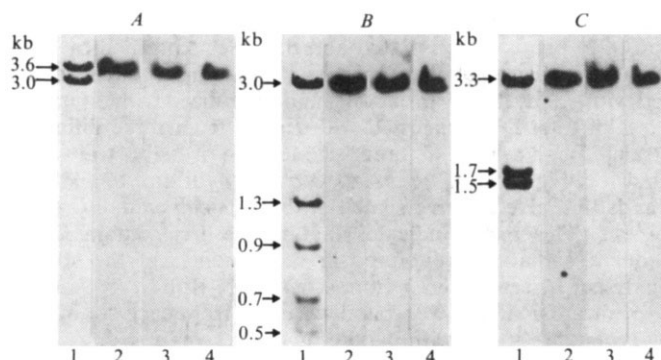


Fig. 3 Regions of homology between *R. phaseoli* and *K. pneumoniae* *nif* genes. *EcoRI* insert from pSA30 was digested with *HindIII* (A), *PstI* (B) or *SalI* (C) restriction endonucleases. Fragments were separated on 1.5% agarose gels, stained with ethidium bromide and blotted onto nitrocellulose filters. Ethidium bromide staining of each digestion is shown in lanes 1. Blots were hybridized with *EcoRI* inserts from plasmids pCQ15 (lanes 2), pCQ12 (lanes 3) or pCQ23 (lanes 4). Hybridizations were performed in 50% formamide as described in Fig. 1 legend using 5×10^5 c.p.m. per lane. Autoradiography was performed at -70°C for 12 h in the presence of intensifying screens. Arrows indicate the size in kilobases of each fragment according to a mixture of λ phage DNA digested with *HindIII* and Φ X174RF phage DNA digested with *HaeIII*, which was run in the same gel.

have hybridized the pSA30, as well as the three different *R. phaseoli* (a, b and c), *EcoRI* inserts to blots of agarose gels of plasmid preparations¹⁶. As shown in Fig. 4, in each strain the four clones hybridized with a single plasmid; however the size of the plasmid varied between strains (approximate molecular weight of plasmid 220×10^6 in strain CFN-Rph5, 170×10^6 in strain CFN-Rph42, 170×10^6 in strain CFN-Rph215 and 150×10^6 in strain CIAT281). It thus seems that the DNA sequences being studied are located on a unique large plasmid in each strain. However, we cannot rule out the presence of cross-hybridizing chromosomal sequences. The hybridization signal present in the lower region of the gels could either be due to chromosomal sequences or to sequences derived from broken plasmids.

Thus we have found that *R. phaseoli* strains have a characteristic pattern of *nif* genes organization including reiterations of DNA sequences. This suggests that there is a relationship between the cross-inoculation group of a strain of *Rhizobium* and the organization of its *nif* genes. The analysis of micro-

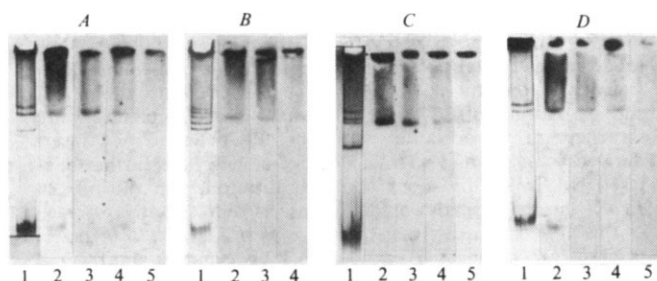


Fig. 4 Hybridization of *R. phaseoli* *nif* genes with plasmids from different strains. Plasmids from strains CFN-Rph5 (A), CFN-Rph42 (B), CFN-Rph215 (C) and CIAT281 (D), were separated on 0.7% agarose gels according to the Eckhardt procedure¹⁶. Gels were stained with ethidium bromide (lanes 1) and blotted onto nitrocellulose filters. Blots were hybridized with ^{32}P -labelled *EcoRI* inserts from plasmids pCQ15 (lanes 2), pCQ12 (lanes 3), pCQ23 (lanes 4) and pSA30 (lanes 5) using 10^6 c.p.m. of probe DNA per lane. Hybridization was performed in 50% formamide as described in Fig. 1 legend and autoradiographies were exposed for 48 h in the presence of intensifying screens. The approximate molecular weight of the hybridizing plasmids mentioned in the text, was obtained from the previously determined molecular weight of the plasmids from strain T3 of *R. leguminosarum*¹, which was run as marker in parallel slots of each gel.

heterogeneity in different clones and their adjacent DNA regions will help determine the actual number and length of the reiterated regions. Stable gene duplications are not a common feature of prokaryotes¹⁹. The finding of naturally occurring *nif* gene reiterations in *R. phaseoli* strains of different geographical origins indicates that they have been preserved during evolution and suggests that they have a significant role.

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Visual transduction in retinal rods of the monkey *Macaca fascicularis*

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The small size and fragility of primate photoreceptors have impeded study of their neural signals by single cell recording. Although physiological information is meagre, psychophysical experiments indicate remarkable performance, including single photon detection by human rods¹. What electrical change results from absorption of a quantum? How do the photoreceptors themselves contribute to the sensitivity and temporal resolution of the primate visual system? We report here a study of transduction in single rods of the cynomolgus monkey. From results of colour matching tests² and microspectrophotometry³ the light receptors of the cynomolgus monkey are thought to be similar to those of man. We have determined the size and shape of the quantal response, and related parameters of the transduction mechanism. These measurements provide a physiological basis for the single photon detection, saturation, and limited temporal resolution characteristic of rod behaviour in psychophysical experiments. Spectral sensitivity measurements over the entire visible region extend previous determinations of the spectral absorption of rhodopsin, and allow assessment of the factors that determine human scotopic sensitivity.

Eyes were obtained from donors in heart-lung transplant experiments, and membrane current was recorded from single rods using suction electrodes^{4,5}. The animals were anaesthetized with ketamine-pentobarbital and enucleation was performed while circulation of the eye was still intact. A black occluder was placed over the cornea 20 min before enucleation; subsequently dark adaptation was preserved by the use of light-tight boxes and IR/visible image converters. After isolation the retina was chopped into pieces about 0.2-0.5 mm wide. The Locke's solution in the recording chamber was continuously oxygenated, and contained: 140 mM Na⁺, 3.6 mM K⁺,

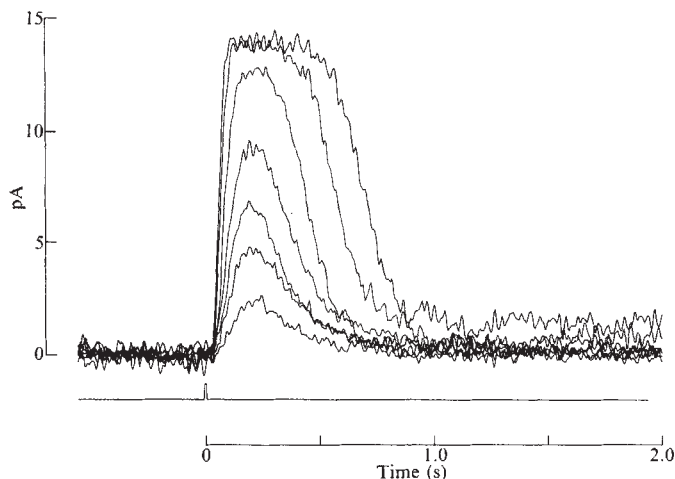


Fig. 1 Family of superimposed responses to brief flashes of increasing intensity. Outer segment current relative to the dark value plotted as a function of time after the 10-ms flash. Outward current upwards, stimulus timing shown by flash monitor below the records. Lower traces averaged from up to five sweeps, uppermost trace is a single sweep. Flash photon densities increased by factors of approximately two between 3.9 and 172 photons μm^{-2} at 501 nm. Temperature, 34 °C.

1.2 mM Ca^{2+} , 2.4 mM Mg^{2+} , 151 mM Cl^- , 3 mM HEPES buffer (pH 7.4), 10 mM glucose. The solution was warmed to $\sim 37^\circ\text{C}$ by passing current through an insulated heating wire in the chamber. Diffuse light stimuli, plane-polarized for optimal absorption in the rhodopsin^{3,6}, were applied at right-angles to the rod axis.

Well-prepared retinal pieces bristled with straight rod outer segments about 25 μm long. Rod outer segments near the edges of the pieces were studied, so that absorption and scatter by other outer segments could be avoided. When drawn into the suction electrode, a single outer segment showed a steady inward dark current^{5,7} of up to 18 pA. Flashes of increasing

intensity progressively reduced the dark current (Fig. 1). Parameters of the flash response (501 nm) from seven cells were (mean $\pm$ s.d.): sensitivity, 0.66 ± 0.47 pA photon⁻¹ μm^2 ; time to peak dim flash response, 254 ± 24 ms; half-saturating flash intensity, 15 ± 4 photons μm^{-2} ; saturating response amplitude, 12 ± 3 pA. The shape of the dim flash response was usually well fitted by equations^{5,8} for the output of a chain of five or six identical low-pass filters of time constant 50–60 ms.

An outer segment 25 μm long and 2 μm in diameter, with a pigment density^{3,6} of $0.016 \mu\text{m}^{-1}$ should have an effective collecting area^{9,10} of $1.9 \mu\text{m}^2$ for transversely polarized light. This, together with the half-saturating flash photon density, implies that approximately 30 photoisomerizations reduce the dark current by half. The same number of isomerizations half-saturates sensitive toad rods^{3,11}, even though their outer segments are twice as long. This suggests that in primate rods the longitudinal spread of activation¹¹ is more restricted, perhaps because diffusion of the internal transmitter is limited by the shorter time scale of the flash response.

A steady background light that gave about 150 isomerizations per s reduced the flash sensitivity to half the dark-adapted value; at 800 isomerizations per s there was a thousand-fold reduction. These measurements indicate that psychophysical rod saturation^{12,13} reflects a property of the outer segment, but that the gain control underlying Weber–Fechner behaviour¹² is more central.

In Fig. 1 the dimmest flash caused an average of seven isomerizations, and gave an average response of ~ 3 pA, implying a single photon effect of 0.4 pA. Fluctuations in the responses to dim lights were consistent with an underlying event of this order of magnitude. From the dim step responses in Fig. 2a, the average estimated peak quantal amplitude was 0.45 pA. This value was calculated from the ratio of variance increase in light to mean response amplitude, using a shape factor¹⁴ of 1.43 and assuming that the light response consists of the linear superposition of a random, independent series of quantal events. The average value for effective collecting area from the same trials was $2.2 \mu\text{m}^2$. The lower histogram in Fig. 2b gives the amplitudes of a series of dim flash responses from the same

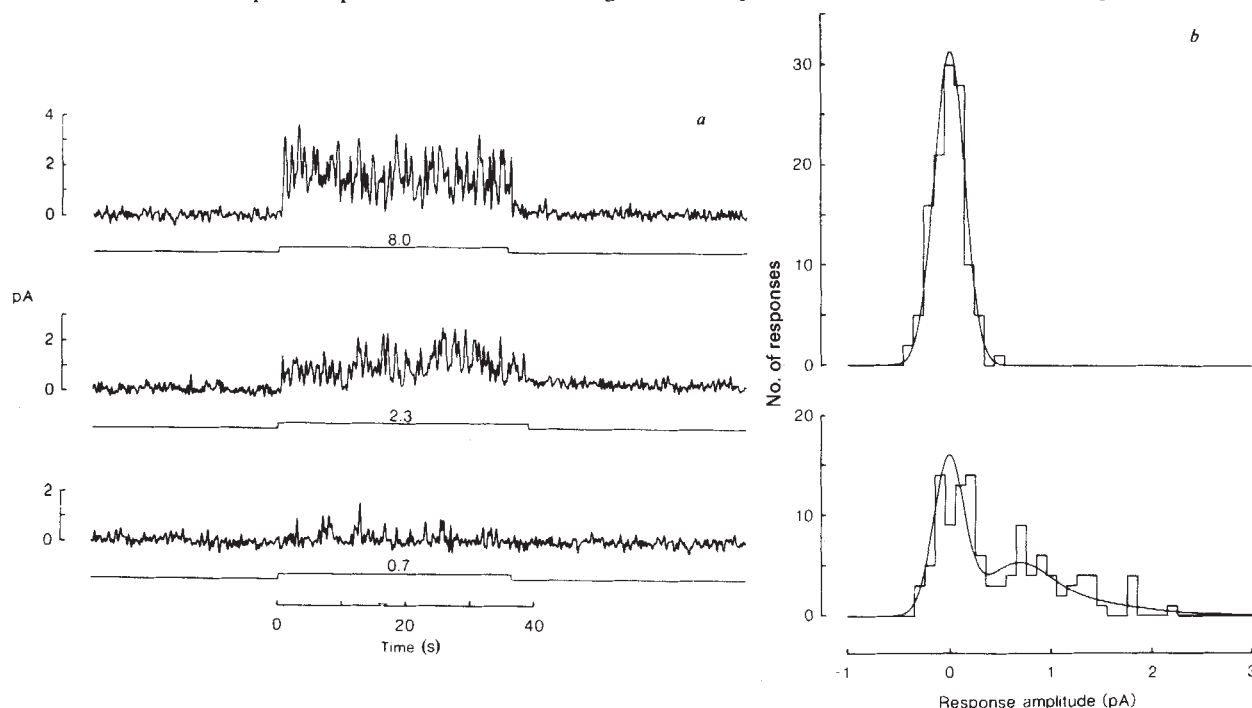


Fig. 2 Photon noise and the quantal response. Traces in *a* show responses to long steps of increasing intensity. Numbers above the light monitor traces give photons $\mu\text{m}^{-2} \text{s}^{-1}$ at 501 nm. Mean responses and variance increases were, from below upwards: 0.162 pA and 0.059 pA²; 0.938 pA and 0.278 pA²; 1.68 pA and 0.494 pA². Lower histogram in *b* shows amplitudes of a series of 116 responses to dim flashes (0.597 photons μm^{-2}) presented to the same rod; mean 0.47 pA, variance 0.32 pA². Upper histogram from 118 sweeps in darkness, fitted by a Gaussian with $\sigma = 0.15$ pA (smooth curve). The continuous curve in the lower histogram was calculated as described in the text. Integration time of flash response, 300 ms; temperature, 36.6 °C; saturating response, 10 pA.

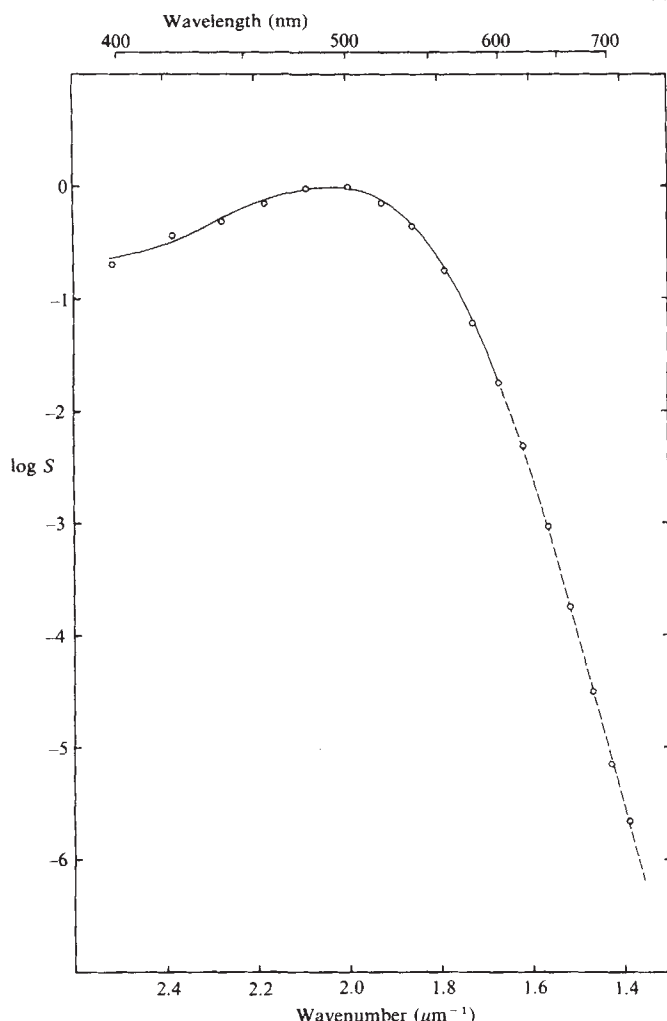


Fig. 3 Spectral sensitivity. Points are average values of log₁₀ relative quantum sensitivity from 10 rods of three monkeys. The solid portion of the curve is the new Dartnall nomogram¹⁸ for a rhodopsin of peak absorption at wavenumber 2.037 μm⁻¹ (491 nm wavelength); the dotted portion of the curve was drawn by eye. Average s.d. of a point was 0.06 log₁₀ unit. Sensitivity was determined from reciprocal photon density to elicit a constant electrical response⁹.

cell, while the histogram above is from a series of interleaved sweeps in darkness. A smooth Gaussian version of the dark noise, scaled to fit the zero peak in the flash histogram, gave an estimated 58 failures in 116 trials. As the occurrence of responses should be Poisson-distributed, the expected number of responses per trial is 0.69; for the mean amplitude of 0.47 pA this gives a quantal amplitude of 0.68 pA. The smooth curve near the flash response histogram was drawn using this value of mean quantal amplitude and a dispersion of 0.3 pA, a baseline dispersion of 0.15 pA (as for the dark histogram above), and with the occurrences of 0, 1, 2, etc. responses given by the Poisson distribution with 0.70 events per trial. The collecting area calculated from the flash responses was 1.2 μm². In the same experiment an independent estimate of 0.65 pA for the quantal amplitude was obtained from the ratio of ensemble variance increase and mean response as functions of time after the flash¹⁰. The rough agreement between the three different amplitude estimates is satisfactory and consistent with a photon effect of 0.4–0.7 pA in this cell. In three other cells the quantal amplitude estimated from dim flash series varied between 0.52 and 0.88 pA. We conclude that a single photoisomerization reduced the dark current by 0.5–1.0 pA. A slightly larger event amplitude was found in toad rods¹⁰.

The average spectral sensitivity of ten rods is shown in Fig. 3, where log relative quantum sensitivity is plotted against wavenumber. The sensitivities should be equivalent to the rela-

tive molecular extinction coefficient of rhodopsin, because the light fell on the outer segments transversely, allowing little 'self-screening'. By fitting the Dartnall nomogram (continuous curve) to the experimental points, the wavelength of maximum absorption of rhodopsin in the outer segment is estimated as 491 ± 3 nm. A value of 500.1 ± 1.6 nm was reported from microspectrophotometry on rods of the same species³, while human rhodopsin in solution^{15,16} gave 493 nm and 494 nm. In the far red the spectral sensitivity declined with a slope similar to that of human rod vision; here human sensitivity should be unaffected by absorption in the lens and by self-screening. For the five points between 640 nm and 720 nm the linear regression slope of log *S* on 1/λ was 15.0 μm, compared with 14.8 μm over the same region from Crawford's averaged psychophysical results¹⁷. Crawford's entire curve was in close agreement with the results in Fig. 3 when corrected for a lens absorption of the form tabulated by Wyszecki and Stiles¹⁸, scaled to a density of 1.62 at 400 nm, and a peak axial pigment density of 0.36.

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Reciprocal tonic activation of inspiratory and expiratory motoneurons by chemical drives

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When CO₂ is lowered to abolish spontaneous respiratory movements (hypocapnic apnoea), expiratory motoneurons show a CO₂-dependent, brain stem-mediated tonic discharge. If CO₂ is then increased this tonic discharge progressively intensifies until it is rendered periodic by inspiratory-linked inhibition as respiratory rhythm occurs at a critical level of CO₂ (refs 1–3). Such results were obtained during oxygen breathing (hyperoxia), hence with minimal excitation of peripheral chemoreceptors. Here we report that in the presence of hypocapnic apnoea, graded oxygen lack (hypoxia) causes a graded discharge of inspiratory motoneurons with graded reciprocal inhibition of the tonic expiratory motoneurone discharge; thus stimulation of peripheral chemoreceptors causes an 'inspiratory shift' in the pattern of respiratory motoneurone activation. When O₂ is lowered further (or CO₂ increased), the onset of respiratory rhythm is now expressed as a periodic, expiratory-phased inhibition of the tonic inspiratory motoneurone discharge. This demonstration that the periodic discharge of both inspiratory and expiratory motoneurons is sculpted from an underlying tonic discharge by reciprocal inhibition allows a new conceptual framework to be advanced. This proposes that the function of the rhythm (pattern) generator is not to cause a phasic excitation of respiratory bulbospinal neurones, as generally assumed previously, but to provide a patterned, phasic inhibition of their tonic excitation by chemical stimuli.

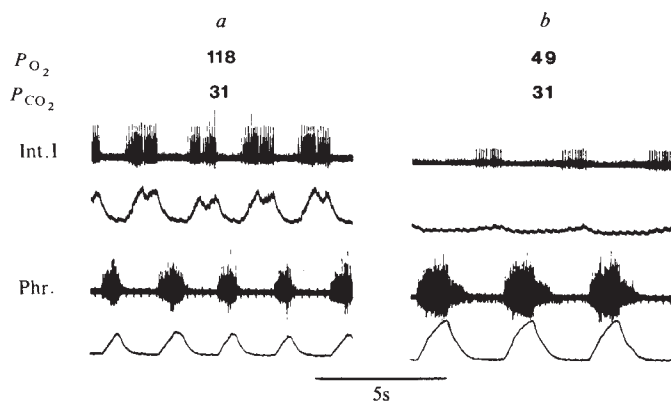


Fig. 1 Steady-state responses of internal intercostal (Int. I) and phrenic (Phr.) motoneurons to normocapnic hypoxia. In each panel the upper two traces are T6 expiratory filament discharge and its corresponding integrated record, and the lower two traces are the C5 Phr. neurogram and corresponding integrated record. Note the increased Phr. discharge with reciprocal decreased Int. I discharge when changing from normoxia (a) to hypoxia (b) at constant P_{aCO_2} .

The present experiments were made on 10 anaesthetized (sodium pentobarbital) cats paralysed with gallamine triethiodide (Flaxedil) and artificially respired (low stroke volume and pump rate 54 per min) with mixtures of O_2 , CO_2 and N_2 to achieve different steady-state chemical drives. The arterial O_2 and CO_2 tensions (P_{aO_2} , P_{aCO_2} , respectively) were measured at 37 °C using a Radiometer system; blood pressure was recorded. Phrenic (Phr.) neurograms were made from the C5 branch and recordings of intercostal motoneurone activity were from the most proximal filaments of the internal (expiratory) and external (inspiratory) intercostal nerves⁴.

Three distinct effects of hypoxia were recognized. The first we designate the 'inspiratory shift'. The records illustrated in Fig. 1a were taken under normal arterial tensions of O_2 and CO_2 (normoxic normocapnia) and show the typical eupnoeic pattern of alternating expiratory (Int. I) and inspiratory (Phr.) motoneurone discharges with their respective integrated versions immediately below. In Fig. 1b hypoxic stimulation of the peripheral chemoreceptors to give a P_{aO_2} of 49 torr (P_{aCO_2} maintained at 31 torr) caused a doubling of peak phrenic activity with a reciprocal reduction of the expiratory activity. The response of the external intercostal motoneurons (not illustrated) was similar to that of the phrenic motoneurons.

The second effect was revealed when graded hypoxia was added during constant hypocapnic apnoea. In Fig. 2a, under normoxic hypocapnia, the expiratory motoneurons exhibited tonic firing together with a low level of phrenic activity as described previously for 'inspiratory biased' preparations². In Fig. 2b, the addition of hypoxia (P_{aO_2} = 44 torr) to stimulate the peripheral chemoreceptors increased the tonic discharge of phrenic motoneurons (and of external intercostal motoneurons) and reciprocally inhibited the expiratory motoneurone discharge. Thus the inspiratory shift due to hypoxia is expressed independently of rhythm generation. Such steady-state, co-activation of inspiratory and expiratory motoneurons and the associated reciprocal inhibitory interaction could usually only be studied within a limited range of arterial gas tensions. This was because if the P_{aO_2} was lowered further, the extra chemical drive caused rhythm to occur; conversely, if P_{aCO_2} was reduced to eliminate this hypoxia-induced rhythm, the expiratory motoneurone discharge was then abolished.

The third effect, a dynamic version of the second, was revealed by first inducing steady-state hypoxic hypocapnic apnoea for 3 min and then stopping artificial ventilation. This was done on the basis that whereas hypoxic stimulation of the peripheral chemoreceptors would increase rapidly, P_{aCO_2} would change only slowly due to the buffering capacity of the blood.

Furthermore, this procedure allowed the interaction of the tonic discharges to be examined without the complication of their modulation by lung or chest wall afferents (see ref. 2). In Fig. 3, for clarity only the 'integrated' records are presented. In Fig. 3a, before stopping the pump (↓) with P_{aO_2} = 29 torr and P_{aCO_2} = 15 torr, there was tonic co-activation of inspiratory and expiratory motoneurons. Within 7 s of stopping the pump, a progressive increase in the inspiratory discharge occurred with reciprocal inhibition of the tonic expiratory activity. At 35 s (P_{aO_2} = 15 torr and P_{aCO_2} = 18 torr), the onset of rhythm is indicated as an abrupt reduction of the tonic inspiratory activity and a reciprocal increase in expiratory activity, two cycles being completed before the pump was started ↑. Note that about 10 s before rhythm occurred, the decline in expiratory activity slowed, halted and then reversed to a progressive increase, this change being accompanied by a slowed rate of rise of inspiratory motoneurone activity. Evidently, two opposing tonic influences were operating (as also seen in Fig. 2) but now with different time courses, as we confirmed by selective elimination of the one due to peripheral chemoreceptor stimulation, leaving intact the other due to central chemosensitivity to CO_2 .

Following denervation of the peripheral chemoreceptors (Fig. 3b) there was no early activation of inspiratory motoneurons; instead, only the central action of CO_2 consisting initially of a slow, then rapidly augmenting discharge of expiratory motoneurons (see refs 1, 2) which peaked at 24 torr. Clearly, this centrally mediated expiratory dominant excitation at low CO_2 had opposed the inspiratory shift due to peripheral chemoreceptor stimulation (Fig. 3a). Eventually (see Fig. 3b), the balance of CO_2 -dependent tonic excitation was restored as the continued increase in CO_2 caused the inspiratory motoneurone discharge to increase and with this a reciprocal reduction in expiratory activity.

These results show that in the absence of rhythm, chemical respiratory stimuli are fully expressed as tonic excitations of respiratory motoneurons. Then the balance of inspiratory and expiratory motoneurone discharge reflects the balance of central (expiratory dominant at low CO_2) and peripheral (inspiratory dominant) chemoreceptor stimulation. However, if there is tonic co-excitation of inspiratory and expiratory motoneurons there must also be corresponding mutual, reciprocal postsynaptic inhibition. This follows from the fact that during rhythm generation, the central respiratory drive potentials of respiratory motoneurons (recorded intracellularly) show a phase of postsynaptic inhibition related directly to the intensity of the excitatory synaptic drive to the antagonistic motoneurons^{5,6}. When rhythm is abolished such mutual

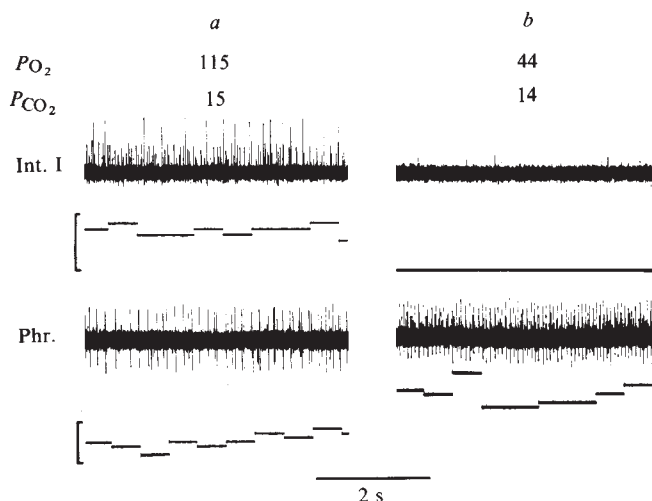


Fig. 2 Steady-state responses of Int. I and Phr. motoneurons to isocapnic hypoxia during constant hypocapnic apnoea. Note the increased tonic Phr. discharge with reciprocal decreased Int. I discharge when changing from normoxia (a) to hypoxia (b). Abbreviations and traces same as in Fig. 1.

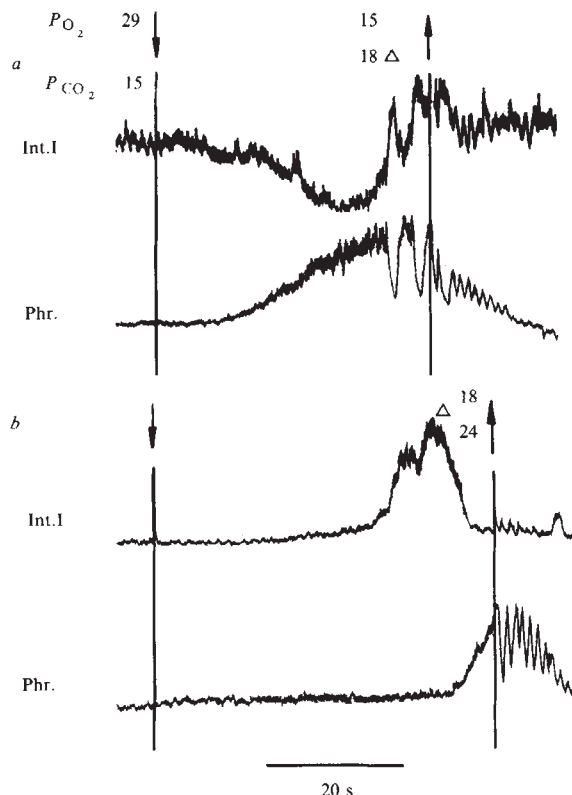


Fig. 3 Response of internal intercostal and phrenic motoneurons to stopping the respiratory pump, starting with steady-state hypoxic hypocapnic apnoea. *a*, Control; *b*, post-denervation of arterial peripheral chemoreceptors. Traces are integrated records for Int. I (T. 11) discharge and C5 Phr. neurogram. Arterial blood gases in torr (mm Hg). The pump was stopped at the downward arrow and restarted at the upward arrow. Abbreviations are the same as in Fig. 1.

inhibition must clearly have an important role in determining the overall balance of inspiratory and expiratory motoneuron discharge.

What can be said about the relation of these tonic activities to the generation of periodic activity? Figure 3*a* shows that with hypoxic hypocapnia, the occurrence of rhythm, due to the combined effects of increasing CO₂ and hypoxia, is expressed as a phasic burst of expiratory activity reciprocal to the phasic inhibition of inspiratory motoneurons. Yet previously it had been shown that in hypocapnic apnoea the occurrence of rhythm is expressed as a phasic inhibition of tonic expiratory activity. However, as seen in Fig. 3, the peak of the expiratory burst before peripheral chemoreceptor denervation (*a*) reaches the same tonic discharge level that after denervation (*b*) is due only to the central action of CO₂. Thus, before rhythm started the excitatory synaptic drive to the expiratory motoneurons coexisted with the drive to the inspiratory motoneurons, each counteracted through mutual reciprocal inhibition. The role of the rhythm generator can then be understood as a mechanism which we propose alternately inhibits the inspiratory and expiratory bulbospinal neurones, thus allowing the disinhibited pathway to express the intensity and balance of the prevailing chemical drives.

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Heterogeneous sensitivity of cultured dorsal root ganglion neurones to opioid peptides selective for μ - and δ -opiate receptors

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Opiate-mediated analgesia at the spinal level is thought to involve opiates binding to opiate receptors on primary afferent terminals¹⁻³ resulting in a selective depression of neurotransmitter release⁴⁻⁹. Multiple opiate receptor types have been distinguished¹¹⁻²² and μ - and δ -opiate receptors, originally described by Kosterlitz *et al.*^{17,18,22}, have been demonstrated on primary afferent terminals¹ but the correspondence of these opiate receptors to opiate-mediated depression of transmitter release is unclear. However, opiates binding to receptors present on individual somata of the dorsal root ganglion (DRG) neurones in dissociated cell culture have been reported to reduce the duration and amplitude of calcium-dependent action potentials^{8,10}. Therefore these opiate receptors might have a function similar to those on primary afferent terminals where a decrease in calcium entry would be correlated with a decrease in transmitter release^{23,24}. We have now studied the response of DRG neurones to opiate agonists with different affinity for μ - or δ -receptors and our results suggest that both receptor types can mediate decrease in somatic calcium-dependent action potentials but that there is a variable proportion of μ - and δ -receptors on DRG neurones.

For these studies we selected the opioid peptides morphiceptin and leucine-enkephalin. Leucine-enkephalin (Leu-enkephalin) is about 1,000-fold more potent than morphiceptin at δ -receptors whereas the two ligands are approximately equipotent at μ -receptors^{11,17,18,22,29}. Preparation of mouse spinal cord and DRG co-cultures and electrophysiological methods were as previously described²⁸. DRG neurones were impaled with 4 M potassium acetate-filled micropipettes (20–40 M Ω), and somatic calcium-dependent action potentials were evoked from resting membrane potential with 100- μ s depolarizing current pulses at a frequency of 4 per min. Action potential durations were generally between 7 and 20 ms due to the addition of 5 mM calcium and 5 mM tetraethylammonium chloride (TEA) to the Tris-HCl (15 mM) buffered experimental medium. The medium (pH 7.2, 320 mOsm) also contained (mM): NaCl, 137; KCl, 5.3; MgCl₂, 0.8; and glucose, 5.6. DRG neurone somatic action potentials are dependent on both sodium and calcium^{25-27,30}. TEA, which decreases voltage-dependent potassium conductance³¹, enhanced the calcium component of DRG neurone action potentials. In TEA-containing medium, DRG neurone action potentials have a sodium component occupying approximately the first millisecond with the remaining broad plateau dependent on calcium²⁶. Preliminary investigation³² was performed using superfusion of medium containing 18 mM TEA. The lower TEA concentrations used in the presently described experiments increased the viability of DRG neurones and increased the stability of action potential duration.

For experiments, aliquots of morphiceptin (Peninsula) and Leu-enkephalin (Calbiochem-Behring) dissolved in distilled water at 1 mM and frozen in plastic tubes were thawed and serially diluted in experimental medium to appropriate concentrations. Morphiceptin and Leu-enkephalin were applied to single neurones by pressure ejection (0.5–2.0 p.s.i.) from micropipettes with tip diameters of 2–5 μ m positioned $\sim$ 5 μ m from the DRG neurone. Opioid peptide application was for 1 s and was delivered 3–4 s before evoking an action potential. In these

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conditions, pressure ejection of opioid peptide-free medium did not decrease the calcium component of action potentials. Additional micropipettes (up to three) with tip diameters of 15–25 μm were used to apply naloxone (Endo) by diffusion when positioned 10–15 μm from DRG neurones. The procedure followed was first to obtain a control response following application of opioid peptide. A naloxone-containing micropipette was then positioned near the DRG neurone, and the antagonist allowed to diffuse into the medium surrounding the cell for 1 min before reapplication of opioid peptide. Finally, the naloxone containing micropipette was removed from its position near the DRG neurone, and the opioid peptide was re-applied. Thus, single DRGs could be characterized in terms of sensitivity to morphiceptin and Leu-enkephalin as well as naloxone concentrations required for antagonism.

In initial studies with morphiceptin, we determined that the opioid peptide decreased DRG neurone somatic calcium-dependent action potential duration dose dependently and over a concentration range similar to that of Leu-enkephalin (100 nM–10 μM)¹⁰. As with Leu-enkephalin, DRG neurone sensitivity to morphiceptin varied considerably across cells with shortening of calcium-dependent action potentials by 1 μM ranging over 2–65%. Comparable maximum decreases in action potential duration were obtained for 1 μM Leu-enkephalin and morphiceptin.

We then compared the actions of morphiceptin and Leu-enkephalin at equimolar concentrations (over 500 nM–5 μM) on somatic calcium-dependent action potentials in single DRG neurones ($n = 133$ of which 98 responded). Opioid peptide effects on action potentials ranged from Leu-enkephalin producing large decreases and morphiceptin not affecting the calcium component of action potentials (Fig. 1*ai*) to equal reduction of action potential duration by both opioid peptides (Fig. 1*aii*). Of the 98 DRG neurones that responded to morphiceptin or Leu-enkephalin, 42 had decreases in action potential duration of at least 20%. Of these 42 neurones, the distribution of relative DRG neurone response to morphiceptin and Leu-enkephalin was as follows (Fig. 1*b*): four DRG neurones responded only to Leu-enkephalin but not to morphiceptin, 30 responded to both peptides but better to Leu-enkephalin than to morphiceptin, five responded equally well to both peptides, and only three DRG neurones responded slightly better to morphiceptin than to Leu-enkephalin (Fig. 1*b*). The distribution of relative response of individual DRG neurones to morphiceptin and Leu-enkephalin was not altered by inclusion of the 56 neurones which showed small responses to the opioid peptides.

DRG neurones characterized by differential sensitivity to morphiceptin and Leu-enkephalin were additionally tested for sensitivity of the opioid peptide actions to naloxone antagonism. Naloxone antagonized decreases in calcium-dependent action potential duration dose dependently with reliable blockade of 500 nM to 5 μM morphiceptin responses produced by naloxone concentrations as low as 5–10 nM (Fig. 2*ai*; *b*, closed triangles). Responses to 500 nM to 5 μM morphiceptin were partially attenuated by naloxone application at a concentration 1/1,000th the morphiceptin concentrations ($n = 5$), almost entirely abolished by naloxone concentrations 1/100th of the morphiceptin concentration ($n = 10$), and completely reversed by naloxone at 1/10th of the morphiceptin concentration ($n = 13$) (Fig. 2*b*, closed circles). However, naloxone sensitivity of Leu-enkephalin mediated decreases in somatic calcium-dependent action potential duration was variable with responses to 200 nM–1 μM Leu-enkephalin reversed by 5–10 nM naloxone in some DRG neurones (Fig. 2*ai*; *b*, closed circles) but requiring 1 μM naloxone for antagonism in other DRG neurones (Fig. 2*aii*; *b*, open circles). The sensitivity of Leu-enkephalin responses to naloxone antagonism correlated with DRG neurone responsiveness to morphiceptin. Leu-enkephalin responses were reversed by low concentrations of naloxone in DRG neurones which responded well to morphiceptin (Fig. 2*ai*; *b*, closed circles), with antagonism occurring at a ratio of

naloxone concentration to opioid peptide concentration of 1:10 ($n = 2$) and 1:100 ($n = 3$) (Fig. 2*b*). In contrast, high concentrations of naloxone were required for antagonism of Leu-enkephalin responses in DRG neurones which responded poorly to morphiceptin (Fig. 2*aii*; *b*, open circles). Naloxone only slightly attenuated Leu-enkephalin responses when applied at a concentration 1/100th ($n = 6$) or 1/10th ($n = 6$) the Leu-enkephalin concentration, moderately attenuated responses when applied at a concentration equal to the peptide concentration ($n = 10$), and totally abolished Leu-enkephalin responses when the naloxone concentration applied exceeded the opioid peptide concentration ($n = 8$) (Fig. 2*b*, open circles).

We observed a subpopulation of DRG neurones which responded well to opioid peptides which may correlate with highly specific opiate effects on sensory transmission. In neurones which responded, the pattern of response to morphiceptin and Leu-enkephalin was heterogeneous. Morphiceptin and Leu-enkephalin decreased the duration and amplitude of somatic calcium-dependent action potentials equipotently in a proportion of DRG neurones, consistent with mediation by μ -receptors. Two additional findings support mediation by μ -receptors. First, large decreases in DRG neurone somatic calcium-dependent action potential duration were produced by 1 μM morphiceptin, a concentration more than 1 log unit below the half-maximal effective dose in the δ -receptor-predominated mouse vas deferens¹¹. Second, DRG neurones which responded equipotently to morphiceptin and Leu-enkephalin were highly sensitive to naloxone antagonism. In contrast, a proportion of

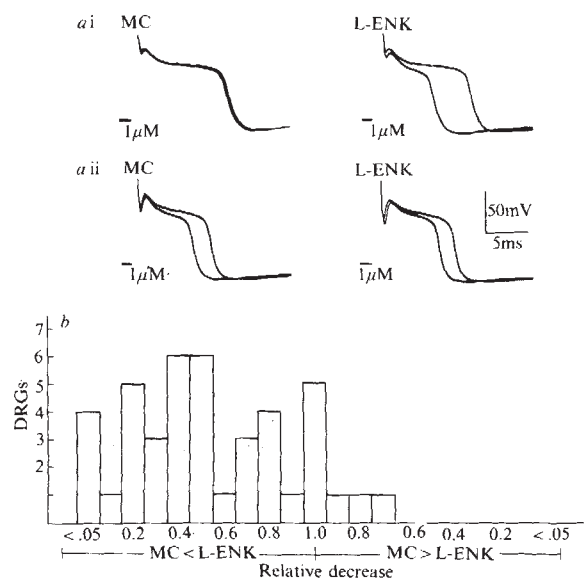


Fig. 1 Heterogeneous response pattern of DRG neurones to morphiceptin and Leu-enkephalin. *a*, *ai* and *aii* illustrate heterogeneous response pattern to morphiceptin (MC) and Leu-enkephalin (L-ENK) observed on DRG neurone somatic calcium-dependent action potentials. Action potentials were evoked from resting membrane potential at 15-s intervals by depolarizing current pulses. In this and the following figure, data shown are superimposed action potentials evoked before (longer duration action potential) and after (shorter duration action potential) opioid peptide application. In all cases action potential duration gradually returned to baseline over 1–2 min. Data shown in *ai* and *aii* are successive recordings from two DRG neurones in the same culture using the same opioid peptide-containing micropipettes. Care was taken to position the morphiceptin- and Leu-enkephalin-containing micropipettes at similar positions near DRG neurones. *b*, Summary of data comparing potency of morphiceptin and Leu-enkephalin on 42 DRG neurones each of which responded with a decrease in action potential duration of at least 20% to Leu-enkephalin or morphiceptin. Data are plotted as relative decrease in action potential duration produced by morphiceptin and Leu-enkephalin on single DRG neurones. A value of 1.0 indicates that morphiceptin and Leu-enkephalin decreased action potential duration equipotently ($n = 5$). To the left of 1.0, morphiceptin was less potent than Leu-enkephalin in decreasing action potential duration with the fraction being DRG neurone response to morphiceptin divided by the response to Leu-enkephalin. To the right of 1.0, morphiceptin was more potent than Leu-enkephalin, and the fractions represent DRG neurone response to Leu-enkephalin divided by the response to morphiceptin.

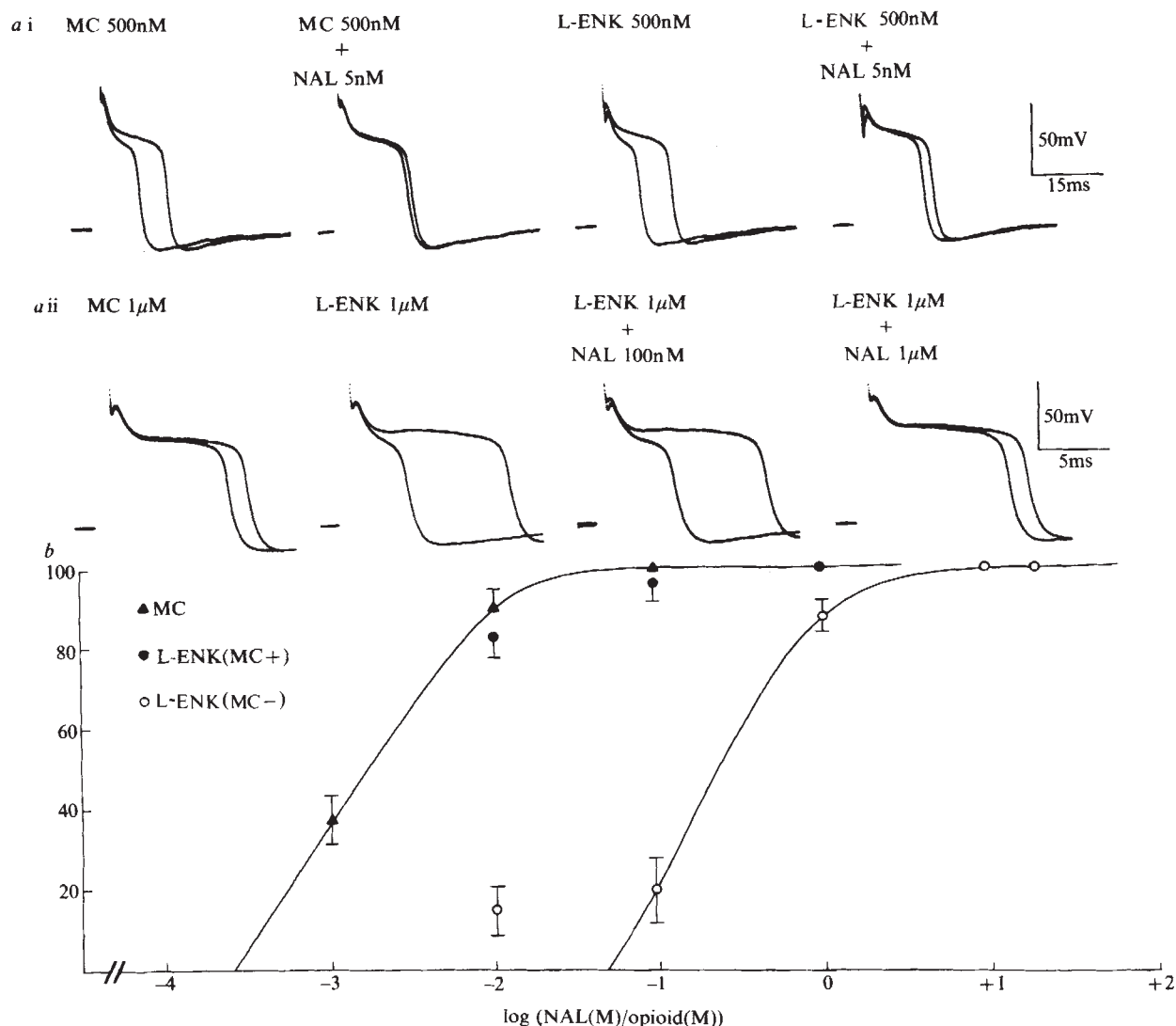


Fig. 2 Morphine-induced decreases in calcium-dependent action potential duration were highly sensitive to naloxone antagonism while higher naloxone concentrations were required to reverse Leu-enkephalin produced decreases of action potential duration in DRG neurones which did not respond to morphine. Single DRG neurones were characterized in terms of sensitivity to Leu-enkephalin and morphine and then the sensitivity of the opioid peptide responses to antagonism by naloxone was tested. *a*, *ai* and *a ii* illustrate DRG neurones with a different response pattern to Leu-enkephalin and morphine and with opioid peptide responses which had differential naloxone sensitivity. *ai*, A DRG neurone which responded approximately equally well to morphine and Leu-enkephalin. Both morphine and Leu-enkephalin responses were highly sensitive to naloxone antagonism. *a ii*, A DRG neurone which responded well only to Leu-enkephalin but not morphine. Antagonism of Leu-enkephalin response required higher naloxone concentration. *b*, Summary of sensitivity to naloxone antagonism of Leu-enkephalin and morphine responses. The ordinate is the per cent decrease in response to the opioid peptides produced by naloxone (NAL). The abscissa is the log of the ratio of naloxone to opioid peptide concentration. For example, a log (NAL/OPIOID) of zero indicates equimolar concentrations of naloxone and opioid peptides, a negative one indicates naloxone at 1/10th the opioid peptide concentration, and a positive one indicates naloxone at 10 times the opioid peptide concentration. Morphine and Leu-enkephalin concentrations ranged over 200 nM–5 μM. ▲, Morphine responses, highly sensitive to naloxone antagonism with almost 100% antagonism at a naloxone to morphine concentration ratio of 0.01. Sensitivity of Leu-enkephalin responses to antagonism by naloxone was correlated with DRG neurone responsiveness to morphine. ●, Leu-enkephalin responses in DRG neurones in which the ratio of response to morphine to response to Leu-enkephalin was at least 0.75 (MC⁺) were approximately as sensitive as morphine responses to naloxone antagonism. In contrast, Leu-enkephalin responses (○) in DRG neurones in which the ratio of response to morphine to response to Leu-enkephalin was less than 0.25 (MC⁻) were about 100-fold less sensitive to naloxone antagonism with 100% antagonism not occurring until naloxone concentrations exceeded Leu-enkephalin concentrations. Bars are standard error of the mean.

DRG neurones were highly sensitive to Leu-enkephalin, did not respond to morphine and required high concentrations of naloxone for antagonism of Leu-enkephalin responses, consistent with the opiate effects being mediated by δ -receptors.

Therefore, our results suggest that both μ - and δ -receptors mediate decreases in somatic calcium-dependent action potentials of DRG neurones. If opiate interaction with these receptors at primary afferents produces a similar effect on calcium entry, the result would be decreased transmitter release. Thus, both μ - and δ -receptors would act to depress transmitter release from primary afferent terminals just as both receptor types have inhibitory actions on myenteric neurones¹⁵. Finally, the heterogeneous pattern of response to morphine and Leu-enkephalin support a variable proportion of μ - and δ -receptors on DRG neurones, suggestive of distinct roles for these recep-

tors in the processing of information at the level of the spinal cord.

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GABA directly affects electrophysiological properties of pituitary pars intermedia cells

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Evidence that the endocrine cells of the pars intermedia of the mammalian pituitary gland secrete not only melanotropins but corticotropins and endorphins¹ heightens interest in the nervous control of these cells. Within the mammalian adenohypophysis the pars intermedia is unique in being directly innervated by neurones whose cell bodies lie in the brain². However, the nature and function of this innervation is poorly understood. A dopaminergic tract has been identified in rats^{3,4}, which appears to have an inhibitory function⁵, and dopamine applied directly to isolated rat pars intermedia cells inhibits both the discharge of spontaneous action potentials⁶ and secretion^{7,8}. In addition, recent immunohistochemical studies in rats indicate that central neurones which apparently contain γ -aminobutyric acid (GABA) also project to the pars intermedia^{9,10}. Here we report that in the same species GABA directly affects the electrophysiological properties of endocrine cells isolated from the pars intermedia and that the ionic and pharmacological characteristics of this action of GABA resemble those encountered at many GABAergic synapses in the central nervous system (CNS). We conclude that the brain can influence the endocrine cells of the pars intermedia directly through GABAergic mechanisms.

Isolated endocrine cells of rat pars intermedia maintained *in vitro* spontaneously discharge action potentials⁶ which are Na spikes with a Ca component¹¹. In such cells, GABA (2×10^{-6} or 1×10^{-5} M) elicited a brief flurry of action potentials of diminishing amplitude, followed by arrest of action potentials for as long as exposure to GABA was maintained (up to 60 s). Shortly after the removal of GABA, action potentials reappeared and increased in amplitude to reach the pre-GABA levels (Fig. 1a). Further analysis (Fig. 1b) showed that GABA rapidly and reversibly depolarized the cells and profoundly reduced membrane resistance—to $11 \pm 2.1\%$ (mean $\pm$ s.e.m., $n = 31$) of the control values with 10^{-4} M GABA. Such changes in membrane potential and resistance explain the ability of

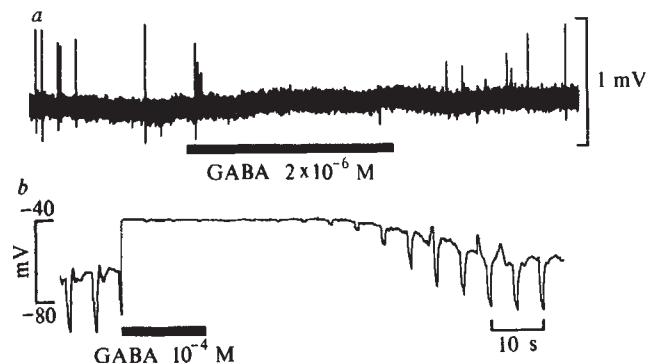


Fig. 1 Typical effects of GABA on electrophysiological properties of isolated pars intermedia cells from rats. *a*, Effect of GABA on spontaneous action potential activity recorded extracellularly. GABA, applied to the cell for the time indicated by the bar, initially caused a brief burst of action potentials followed by cessation of action potential activity. Note that the amplitude of the action potentials wanes during the initial burst and waxes during the recovery from exposure to GABA. *b*, Effect of GABA on membrane potential and resistance recorded intracellularly. In this record, and in those shown in Fig. 2, membrane resistance is assessed at regular intervals (~ 5 s) by passing brief inward (hyperpolarizing) current pulses of constant magnitude. The amplitude of the resulting hyperpolarizing responses thus reflects membrane resistance such that a reduction in amplitude signals a fall in resistance. GABA produced an immediate depolarization accompanied by a profound reduction in membrane resistance which lasted, in this cell, for many seconds after GABA was withdrawn. In other cells recovery from GABA was more rapid (compare with Fig. 2d). The cells used in this and all other experiments were endocrine cells dispersed from the pars intermedia of rat neurointermediate lobes, maintained in short-term culture (<14 days) and recorded from as previously described⁶. The solution in which the cells were bathed during recording contained (mM): NaCl, 150; KCl, 5; CaCl₂, 2; MgCl₂, 1; glucose, 11; HEPES, 5 (pH 7.1). GABA, and all other drugs, were delivered in this solution to the cells by a gravity flow pipette (inner tip diameter about $10 \mu\text{m}$) which effectively perfuses the cell.

GABA to stimulate and arrest spike discharge and reduce spike amplitude.

The collapse of membrane resistance and potential in the pars intermedia cells in response to GABA seems to result from an increased Cl^- conductance. This is indicated by the dependence of the null potential of the GABA response on the extracellular concentration of Cl^- ions (Fig. 2a,b). Such an effect, to increase Cl^- conductance, is now a familiar action of GABA at sites of GABAergic transmission within the brain and spinal cord¹²⁻¹⁴. At many such sites, the Cl^- -dependent responses to GABA are inhibited by bicuculline and potentiated by diazepam¹²⁻¹⁶. We found that these drugs similarly affected the responses of the pars intermedia cells to GABA (Fig. 2c-f). Bicuculline, given with GABA in equimolar amount (10^{-4} M), potently, rapidly and reversibly antagonized the GABA response, both the fall in membrane resistance and membrane potential being greatly abbreviated or abolished (Fig. 2c,d). Even when given in a 10-fold lower concentration (10^{-5} M), bicuculline markedly inhibited the response to GABA (10^{-4} M), although a vestige of the response was then regularly apparent in an early, short-lived (5-15 s) phase of reduced membrane resistance and potential. Diazepam (2×10^{-5} M), by contrast, rapidly and reversibly potentiated responses to GABA. This effect was prominent (Fig. 2e,f) when GABA was used in clearly submaximal concentrations: responses to maximally effective concentrations of GABA were little, if at all, affected by diazepam. Neither bicuculline nor diazepam by itself had any apparent effect on resting membrane potential or resistance.

In functioning as the principal inhibitory transmitter at central synapses, GABA often acts to increase conductance to Cl^- ions. This action can produce two prominent effects¹²⁻¹⁴. First, acting directly on the nerve cell body, GABA can reduce excitability and spontaneous activity. Clearly, there is a parallel here in the behaviour of the pars intermedia cells (Fig. 1a). Second, acting on presynaptic terminals, GABA can diminish the depolarizing stimulus to secretion (the action potential) and thereby reduce transmitter output. Here, again, we obtained

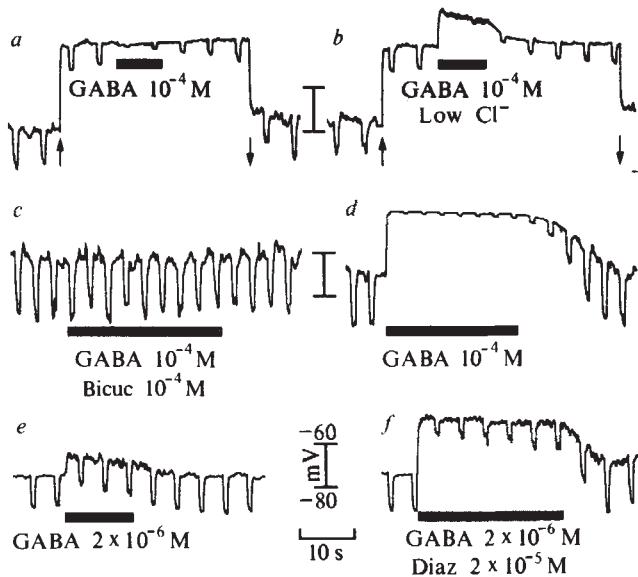


Fig. 2 Chloride-dependence and pharmacological features of the GABA response as revealed by intracellular recording from isolated pars intermedia cells. *a, b*, The influence of Cl^- concentration on the null potential of the GABA response. *a*, Null potential in standard recording solution (see Fig. 1 legend). The cell was depolarized to about -40 mV by a sustained pulse of outward current applied for the time indicated by the arrows. GABA applied during this period caused the usual reduction in membrane resistance but did not cause any depolarization. *b*, Change in null potential on reduction of the external concentration of Cl^- . The same cell in the same conditions as in *a* but perfusion with GABA in low Cl^- solution (150 mM NaCl replaced by 150 mM Na isethionate) again reduces resistance and now causes a depolarization. As this low Cl^- solution alone had no such effect, these observations indicate that increased Cl^- conductance contributes importantly to the GABA response. *c, d*, The inhibitory effect of bicuculline (Bicuc). *c*, GABA administered with bicuculline produces little if any change in membrane potential or resistance. *d*, Subsequent, control, response of the same cell to GABA alone. *e, f*, The potentiating effect of diazepam (Diaz). *e*, A low concentration of GABA produces a slight depolarization and reduction of membrane resistance. *f*, Subsequently, in the same cell, this concentration of GABA given with diazepam causes a much larger depolarization and decrease in membrane resistance.

evidence for parallel behaviour when we studied the effect of GABA on the depolarizing response to excess K^+ . Like neurones, the rat pars intermedia cells possess voltage-dependent Ca^{2+} channels¹¹ whose activation by excess K^+ , for example, induces Ca^{2+} -dependent secretion¹⁷. Our experiments showed that GABA greatly reduced the depolarizing effect of excess K^+ (100 mM) on pars intermedia cells (Fig. 3).

As the endocrine cells of the pars intermedia are derived from Rathke's pouch, which is classically considered to be an invagination of stomodeal ectoderm^{18,19}, it might appear surprising that they should possess, in addition to other neurone-like properties, a GABA receptor-ionophore complex similar to that present at synapses in the CNS^{12-14,16,17}. However, it has been argued that pars intermedia and other adenohypophyseal cells are of neuroectodermal provenance^{20,21}. In any event, our results show that GABA has direct actions on the endocrine cells of the rat pars intermedia whose electrophysiological correlates might be expected to influence hormone output; and indeed experiments on isolated, perfused rat melanotrophs¹⁷ show that GABA has a transient stimulant and then an inhibitory effect on 'basal' MSH secretion and inhibits K-evoked secretion (S. A. Tomiko, P.S.T. & W.W.D., unpublished work).

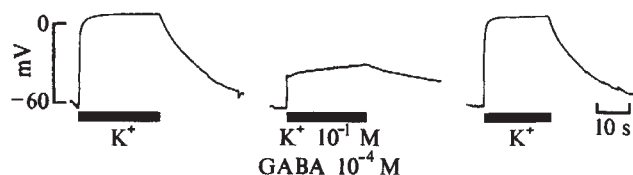


Fig. 3 Reduction of K^+ -induced depolarization by GABA. Three successive responses of a single pars intermedia cell to a high K^+ solution (100 mM NaCl replaced by 100 mM KCl) given alone or accompanied by GABA.

Our evidence, taken together with the immunohistochemical demonstration of an abundant innervation of the region by GABAergic fibres of central nervous origin^{9,10}, permits the conclusion that the brain can influence the endocrine cells of the pars intermedia through GABAergic mechanisms. Whether or not the GABAergic innervation can also act indirectly to influence the pars intermedia cells by presynaptic actions¹²⁻¹⁴ on neurones of the other types that are present², such as the dopaminergic fibres^{3,4}, is not known, but the occurrence of axo-axonal synapses in the rat pars intermedia⁹ is consistent with this possibility. Although future experiments must define the role of the GABAergic component in the overall control of the endocrine function of the pars intermedia, this component is clearly a new element. Furthermore, the pharmacological similarities between the actions of GABA on pars intermedia cells and on central neurones seems likely to have clinical relevance. Many drugs, including the very widely prescribed anti-anxiety agent diazepam (Valium) and its congeners, which are known to modulate GABAergic mechanisms in the CNS¹⁶, may interfere with the endocrine functions of pars intermedia cells which, *inter alia*, seem to include influences on human fetal development²².

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Serum antibodies that distinguish between the phospho- and dephospho-forms of a phosphoprotein

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Considerable evidence suggests that the actions of many hormones, neurotransmitters, neuromodulators and oncogenic viruses are mediated through regulation of the state of phosphorylation of specific proteins^{1,2}. Many such phosphoproteins have been found, which serve as specific substrates for various classes of protein kinases^{1,2}. We report here the successful preparation of antibodies which are selective for either the dephospho- or the phospho-form of one such phosphoprotein. Thus, we have prepared both dephospho-selective and phospho-selective serum antibodies to G-substrate^{3,4}, a neurone-specific substrate for cyclic GMP-dependent protein kinase, and have used them as the basis of a radioimmunoassay procedure for studying the state of phosphorylation of this protein.

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G-substrate is highly concentrated in the Purkinje cells of the mammalian cerebellum³⁻⁵, as is the cyclic GMP-dependent protein kinase itself⁶⁻⁸. G-substrate has been purified to homogeneity from rabbit cerebellum⁴ and is phosphorylated on threonine residues in two different sites which exhibit a considerable degree of sequence homology⁹. Using a seven-

amino acid synthetic peptide, Arg-Lys-Asp-Thr-Pro-Ala-Leu, which is common to both sites, antibodies were prepared in rabbit following the cross-linking of the antigen to keyhole limpet haemocyanin. Figure 1a shows that this antibody preparation (referred to as dephospho-antibody) precipitated ¹²⁵I-dephospho-G-substrate preferentially to ¹²⁵I-phospho-G-substrate. With a serum dilution of 1,000-fold, ~55% of the dephospho-G-substrate was precipitated, compared with only 6% for the phospho-G-substrate (Fig. 1a).

Rabbit serum antibodies were also prepared using as antigen, G-substrate, purified in the fully phosphorylated form. This antibody preparation (referred to as phospho-antibody) displayed significantly higher affinity for ¹²⁵I-phospho-G-substrate than for ¹²⁵I-dephospho-G-substrate (Fig. 1b); with serum dilutions of 200-fold, ~50% of the phospho-G-substrate was precipitated in conditions in which less than 5% of the dephospho-G-substrate was precipitated.

Non-equilibrium radioimmunoassay procedures were developed for both antibodies using serum dilutions of 750-fold for the dephospho-antibody preparation and 500-fold for the phospho-antibody preparation. Figure 2a shows a representative assay using the dephospho-antibody. When standard dephospho-G-substrate competed with ¹²⁵I-dephospho-G-

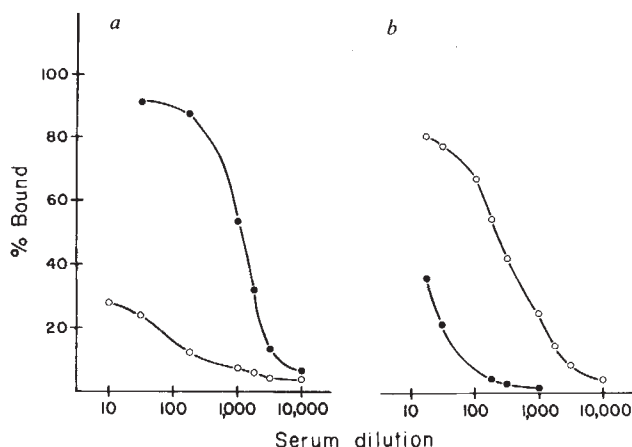


Fig. 1 Immunoprecipitation of dephospho-G-substrate and phospho-G-substrate by dephospho- and phospho-selective antibodies. Dephospho-G-substrate and phospho-G-substrate were purified from rabbit cerebellum essentially by the method of Aswad and Greengard⁴ with additional purification using isoelectric focusing between steps 5 and 6. Antibodies were prepared in female New Zealand rabbits by multiple intradermal injection: Antibodies selective for dephospho-G-substrate were prepared using as antigen a synthetic peptide, Arg-Lys-Asp-Thr-Pro-Ala-Leu, consisting of the seven amino acids surrounding the phosphorylatable threonine in G-substrate. 50 μ mol of the synthetic peptide, prepared as described previously¹³, was conjugated to 20 mg of keyhole limpet haemocyanin (KLH) in 2 ml using 1-ethyl-3-(3-dimethyl aminopropyl)carbodiimide-HCl as described elsewhere¹⁴. 0.3 ml of the KLH-peptide (7.5 μ mol) mixed homogeneously with 0.3 ml of complete Freund's adjuvant was injected on day 1 into each of three rabbits. Subsequent injections were given 14 and 28 days later, respectively, using 0.2 ml KLH-peptide (5 μ mol) mixed with 0.2 ml incomplete Freund's adjuvant, and 0.15 ml KLH-peptide (3.75 μ mol) mixed with 0.15 ml incomplete Freund's adjuvant. Antibodies selective for phospho-G-substrate were prepared using as antigen fully phosphorylated G-substrate. Each of three animals were injected on day 1 with 0.2 mg of phospho-G-substrate (1 mg ml⁻¹ in physiological phosphate-buffered saline) mixed with 0.2 ml of complete Freund's adjuvant. Subsequent injections were given 12 and 24 days later using 0.1 mg phospho-G-substrate in 0.1 ml of incomplete Freund's adjuvant. All animals were bled by cardiac puncture 2 weeks after the third inoculation and immune sera prepared. Blood was allowed to stand overnight at 4 °C, serum decanted and centrifuged at 7,000g for 10 min and aliquots of the supernatant stored frozen at -70 °C. Preimmune serum was obtained from each animal before the first injection. All animals in each group produced selective antibodies. However, only results using serum from the animal with the highest titre in each group are presented. Dephospho-G-substrate and phospho-G-substrate were iodinated using ¹²⁵I-labelled Bolton Hunter Reagent (NEN) as described previously¹⁵. Immunoprecipitation of ¹²⁵I-dephospho-G-substrate or ¹²⁵I-phospho-G-substrate was performed using Protein A-bearing *Staphylococcus aureus* (SAC). Before immunoprecipitation, SAC was prepared as described elsewhere¹⁵, except that the buffer (NET buffer) used contained 150 mM NaCl/15 mM EDTA/50 mM Tris-HCl (pH 7.4)/50 mM NaF/0.02% sodium azide and 1% Nonidet P40. ¹²⁵I-G-substrate was 'precleared' by bringing the volume of ¹²⁵I-G-substrate to 100 μ l with NET buffer and adding 10 μ l of SAC. After incubating for 20 min at 0 °C, the sample was centrifuged and the supernatant containing the precleared ¹²⁵I-G-substrate was removed and diluted to required concentrations (1,000–2,000 c.p.m. μ l⁻¹) in NET buffer. Immunoprecipitation of ¹²⁵I-G-substrate was performed using 96-well microtitre assay plates. 10 μ l of antibody diluted as indicated (in NET buffer), and 10 μ l of ¹²⁵I-G-substrate were added to 80 μ l of NET buffer and the sample incubated for 2 h at 4 °C. 20 μ l of SAC was then added and the sample incubated for a further 15 min before the assay was terminated by centrifugation for 15 min at 1,000g using the Cooke Microtiter system (Dynatech). Supernatants were removed by inversion. The pellets were resuspended and washed with 100 μ l of NET buffer. Radioactivity bound was measured, either directly in the washed pellets, or following SDS-polyacrylamide gel electrophoresis and autoradiography¹⁶, using a γ counter (Micromedex Systems, type 2/200). ●, ¹²⁵I-dephospho-G-substrate; ○, ¹²⁵I-phospho-G-substrate. a, dephospho-antibody; b, phospho-antibody. % Bound was 100 $\times$ the counts precipitated/total G-substrate counts added to each assay. Each data point is the mean of duplicate measurements.

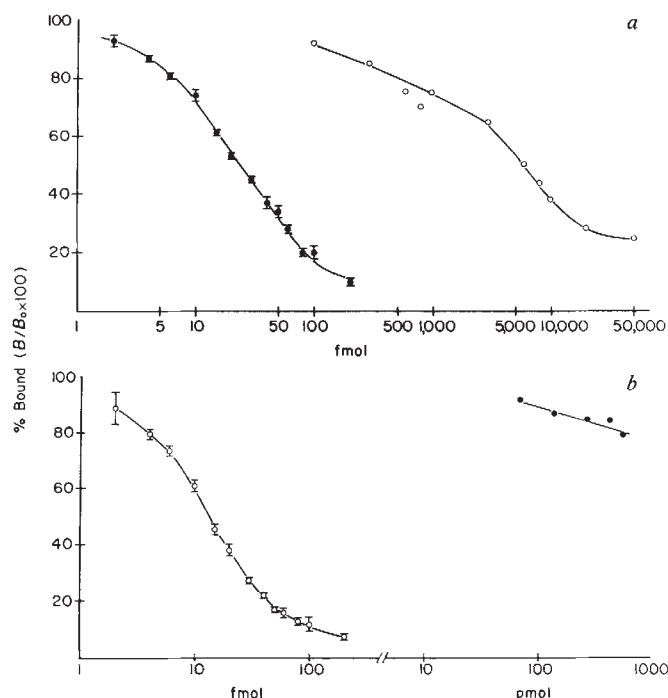


Fig. 2 Radioimmunoassay of dephospho-G-substrate and phospho-G-substrate using dephospho- and phospho-selective antibodies. Materials were prepared as described in Fig. 1 legend. Non-equilibrium radioimmunoassay was performed in 96-well microtitre assay plates at 4 °C. All additions were made by Hamilton repeating dispensers using Hamilton gas-tight fixed needle syringes. Dephospho-antibody was diluted 750 $\times$ and phospho-antibody 500 $\times$. The samples of standard G-substrate to be assayed were brought to a volume of 80 μ l in NET buffer and 10 μ l of antiserum were added. After 30 min, 10 μ l of ¹²⁵I-labelled G-substrate were added and the assay mixture incubated for 3–8 h following which a 10- μ l aliquot of SAC was added. After 15 min, the samples were centrifuged, washed and counted as described in Fig. 1 legend. Standards were assayed in triplicate and where indicated, error bars represent the standard deviation. Blanks were obtained by omission of antibody and standard protein; B_0 was obtained by omission of standard protein. (B_0 was 50–60% of counts added; blanks were <3% of B_0). The plot of B/B_0 against standard G-substrate was not significantly altered by changes in the concentration of ¹²⁵I-G-substrate or the time of incubation. ●, Dephospho-G-substrate; ○, phospho-G-substrate. a, Dephospho-antibody. logit-log transformation of the dephospho-G-substrate data yielded a linear relationship between 4 and 200 fmol, with a slope of -2.4 and a regression coefficient of 0.998. b, Phospho-antibody. logit-log transformation of the phospho-G-substrate data yielded a linear relationship between 2 and 80 fmol, with a slope of -2.6 and a regression coefficient of 0.998.

substrate, 2–200 fmol could be accurately measured. Figure 2a also shows that standard phospho-G-substrate was only 0.4% as effective (at $B/B_0 = 50\%$) in competing with ^{125}I -dephospho-G-substrate using this antibody. Figure 2b shows analogous results using the phospho-antibody. When standard phospho-G-substrate competed with ^{125}I -phospho-G-substrate, 2–80 fmol could be accurately measured. In contrast, even at very high concentrations, dephospho-G-substrate showed almost no competition with ^{125}I -phospho-G-substrate.

Through the use of the radioimmunoassay procedures for the dephospho- and phospho-forms of G-substrate, it was possible to follow the course of the phosphorylation reaction when G-substrate was incubated with cyclic GMP-dependent protein kinase and Mg-ATP. As shown in Fig. 3, the increase in the amount of phospho-G-substrate, determined by radioimmunoassay, was balanced by the decrease in the amount of dephospho-G-substrate, determined by radioimmunoassay. Moreover, when ATP was replaced by ^{32}P -ATP in the reaction mixture, the incorporation of ^{32}P into G-substrate paralleled the formation of phospho-G-substrate determined by radioimmunoassay. Results similar to those shown in Fig. 3 have been

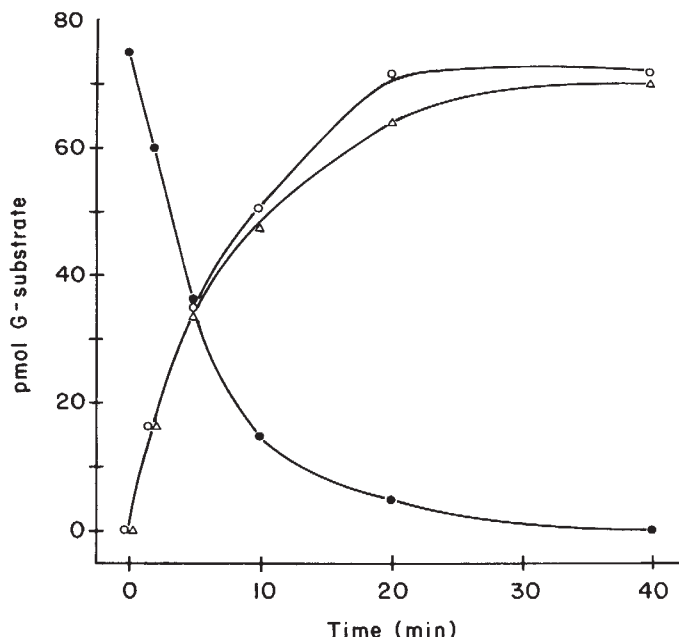


Fig. 3 Measurement of the state of phosphorylation of G-substrate using radioimmunoassay procedures selective for dephospho-G-substrate and phospho-G-substrate. Phosphorylation of G-substrate ($17\text{ }\mu\text{g ml}^{-1}$) was carried out in a total volume of 0.1 ml using a reaction mixture containing (final concentrations): 10 mM HEPES (pH 7.4)/0.3 mM ethylene glycol bis(β -aminoethylether)- N,N,N',N' -tetraacetic acid/1 mM 3-isobutyl-1-methylxanthine/10 mM MgCl_2 /1 μM cyclic GMP and 4 $\mu\text{g ml}^{-1}$ cyclic GMP-dependent protein kinase (purified as described previously¹⁷). The reaction was initiated by the addition of 50 μM ATP and incubation was carried out at 30 °C for the time indicated. The reaction was terminated by the addition of 20 μl of 6 $\times$ NET buffer followed by boiling for 30 s. Each sample was diluted to appropriate concentrations in NET buffer and assayed for both dephospho-G-substrate and phospho-G-substrate by radioimmunoassay, as described in Fig. 2 legend. In each radioimmunoassay, four different volumes between 10 and 80 μl were analysed. In all cases a linear relationship was obtained which paralleled the appropriate standard curve. At each concentration, samples were assayed in duplicate, and the data were extrapolated by graphical analysis. The addition of cyclic GMP, cyclic GMP-dependent protein kinase or Mg-ATP alone, to standard dephospho- or phospho-G-substrate had no effect on the radioimmunoassay curves. Parallel assays were performed in which [γ - ^{32}P]ATP (250–500 c.p.m. pmol^{-1}) replaced ATP. These reactions were terminated by the addition of 10 μl of 0.5 M H_2SO_4 and ^{32}P incorporation was measured using the procedure described for G-substrate⁴. Data for ^{32}P incorporation are the mean of duplicate measurements and represent pmol of G-substrate, correction being made for the fact that phosphorylation occurred on two sites. ●, Dephospho-G-substrate measured by dephospho-radioimmunoassay; ○, phospho-G-substrate measured by phospho-radioimmunoassay; △, phospho-G-substrate measured by ^{32}P incorporation.

obtained using less pure preparations of G-substrate. The method has also been used to measure the state of phosphorylation of G-substrate in slices of rabbit cerebellum incubated in various experimental conditions, and to determine the amount of dephospho- and phospho-G-substrate in the cerebellum of various species (data not shown).

The ability of the dephospho-antibody to distinguish between the dephospho- and phospho-forms of G-substrate is attributable to our using, as the antigen, a short peptide from the region of the molecule containing the phosphorylatable threonine. Results from peptide mapping experiments indicate that the high degree of selectivity of the phospho-antibody is also attributable to the interaction of the antibody preparation with a region of G-substrate at or near the phosphorylated threonine. In these experiments (data not shown), the phospho-antibody was found to immunoprecipitate selectively peptides containing the phosphorylated threonine residue, but not peptides from other regions of the G-substrate molecule. Each phospho-G-substrate antibody preparation exhibited a high degree of selectivity for the phospho-form of G-substrate, indicating that the region around the phosphorylated threonine is highly antigenic. It will be of interest to determine if the same region of the molecule is also antigenic when dephospho-holo-G-substrate is used as the antigen.

In general, it should be possible to prepare dephospho-selective and phospho-selective antibodies for any phosphoprotein. For this purpose, either dephospho- or phosphopeptides, representing the amino acid sequence around the phosphorylatable residue, could be used as the antigen. Such peptides could be prepared either synthetically or by proteolytic digestion of the protein of interest. Alternatively, in the case of phosphoproteins in which the region of the molecule containing the phosphorylatable residue is highly antigenic, as with phospho-G-substrate, the dephospho- or phospho-holo-proteins, themselves, could be used as antigen.

The ability to prepare antibodies which are selective for either the dephosphorylated form or the phosphorylated form of phosphoproteins should be very useful in elucidating the functional significance of such proteins. Such specific antibodies could be used: (1) to inhibit the phosphorylation and dephosphorylation reactions in cell-free systems, (2) to study the state of phosphorylation of the protein in intact cells in response to various physiological and pharmacological manipulations, and (3) to introduce selective antibodies into intact cells as has recently been done with the catalytic subunit of cyclic AMP-dependent protein kinase in *Aplysia* neurones^{10–12}.

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Monoclonal antibodies to circumsporozoite proteins identify the species of malaria parasite in infected mosquitoes

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An understanding of the epidemiology of malaria depends on the accurate identification of the portion of the mosquito vector population carrying infectious sporozoites. This is usually determined by laborious dissection and examination of individual mosquitoes, often in areas where less than 1 per 1,000 mosquitoes contain sporozoites¹. Moreover, it is impossible to identify the species of malaria parasite in the invertebrate host by morphological criteria. Here we describe two sensitive immunoradiometric assays which use monoclonal antibodies to detect circumsporozoite (CS) protein antigens. These tests can detect, identify and quantify sporozoites in individual or pools of mosquitoes; minimal effort is required and testing can be performed long after mosquito collection at sites removed from endemic areas.

Monoclonal antibodies to CS proteins of *Plasmodium berghei*², *Plasmodium knowlesi*³, *Plasmodium vivax* and *Plasmodium falciparum*⁴ were obtained from ascites fluid of hybridoma-bearing mice, and radiolabelled with ¹²⁵I to specific activities of 2–3 × 10⁷ c.p.m. per µg of protein. The first immunoassay, qualitative in nature, was based on the observation that CS proteins have a high avidity for plastic surfaces. Single *Anopheles stephensi* mosquitoes, uninfected or which had fed on *P. berghei* or *P. vivax* gametocyte-containing blood, and *Anopheles dirus*, uninfected or which had fed on *P. knowlesi* or *P. falciparum* gametocyte-containing blood, were killed with ether and placed in wells of plastic microtitre plates; 50 µl of phosphate-buffered saline (PBS) containing 25 µg ml⁻¹ protease inhibitors² were added to the wells. The thorax and head of each mosquito were crushed with a wooden applicator, and the sporozoite antigens extracted by freezing and thawing, followed by heating at 56 °C. The wells were washed with PBS containing 1% bovine serum albumin (BSA) and 0.05% sodium azide, and incubated in the same buffer for 1 h at room temperature. ¹²⁵I-labelled monoclonal antibodies (5 × 10⁴ c.p.m. in 30 µl) were added, and after an additional incubation and several washings, the wells were cut and counted in a γ-counter.

Two types of control were included: uninfected mosquitoes incubated with specific antibodies, and infected mosquitoes incubated with antibodies to a heterologous CS protein. The results (Fig. 1) demonstrate that the population of infected mosquitoes was revealed by the homologous but not the heterologous antibodies. To evaluate the sensitivity of the method, the salivary glands of several mosquitoes from each group were examined by light microscopy. The percentages of infection determined microscopically were always lower or equal to those obtained in the immunoassay.

Some features of this assay make it particularly suitable for use in epidemiological surveys. The assay is reproducible, and has been used with identical results in two laboratories. As shown in Fig. 1, infection with *P. berghei* was detected in mosquitoes that had been killed with ether and kept dry for 3 days. Similar studies with *A. dirus* infected with *P. knowlesi* have shown that dead mosquitoes may be kept dry or frozen (–4 °C) for at least 7 weeks with no significant loss of detectable

sporozoite antigen. Ten individual uninfected dried (7 weeks) mosquitoes showed a range of 3.8–6.8 × 10² c.p.m. while 10 identically treated infected mosquitoes recorded a range of 1.8–6.4 × 10³ c.p.m. Pools of 25 mosquitoes containing a single dried (2 weeks) infected *A. dirus* were equally detectable. Uninfected control pools showed a range of 1.1–1.9 × 10³ c.p.m. while pools with one infected specimen ranged from 2.1 × 10³ to 7.0 × 10³ c.p.m. Note that the hybridomas producing monoclonal antibodies to *P. vivax* and *P. falciparum* were derived from mice immunized with isolates of sporozoites different from those used in the assay. As shown elsewhere, these monoclonal antibodies recognize epitopes common to several geographical isolates of the homologous species⁴.

In many areas, two or three species of malaria parasite infect man. Identification of the species of sporozoite carried by a particular vector would be of considerable interest. A mixture of monoclonal antibodies labelled with two different radioisotopes, for example, ¹²⁵I-anti-*P. vivax* and ¹³¹I-anti-*P. falciparum*, could be used to detect these parasites in the same sample of mosquitoes. This assay, however, is not quantitative, because the plastic wells bear a limited number of sites to bind antigen. Moreover, the efficiency of extraction of CS proteins from the mosquitoes may vary. Therefore, this assay cannot be used to compare the intensities of infection of different batches of *Anopheles*.

For this specific purpose we developed a two-site immunoradiometric assay (IRMA). The wells of plastic plates are first coated with monoclonal antibodies to an epitope of the CS protein, then incubated with crude extracts of sporozoites, and washed. The antigen specifically bound is measured using an excess of radiolabelled monoclonal antibody directed against another epitope of the CS protein. In this type of assay, the counts remaining in the well are directly propor-

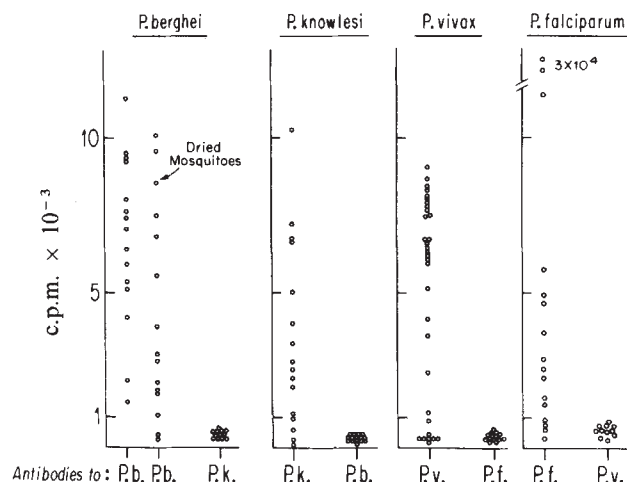


Fig. 1 Detection of malaria infection in individual mosquitoes using monoclonal antibodies to CS proteins. Infected mosquitoes with malaria sporozoites were obtained as described elsewhere (refs 8, 9 for *P. berghei* and *P. knowlesi*, and ref. 4 for *P. vivax* and *P. falciparum*), and placed in wells of a microtitre plate. The thorax and head of each mosquito were crushed, and then the plate was incubated at –70 °C for 5 min and defrosted at room temperature. This procedure was repeated six times. The plate was then heated at 56 °C for 5 min, the wells washed three times with PBS–BSA–sodium azide, filled with the same buffer, and kept for 1 h at room temperature. The wells were emptied, and 30 µl (5 × 10⁴ c.p.m.) of ¹²⁵I-labelled purified monoclonal antibodies were placed in the wells. The plate was incubated for 2 h at room temperature, washed three times with PBS–BSA–azide containing 0.05% Tween-20, and the wells were cut and counted in a γ-counter. An additional control, not shown here, consisted of adding each labelled monoclonal antibody to separate wells containing uninfected mosquitoes. The number of c.p.m. obtained was 163 ± 55, 195 ± 50, 264 ± 49, 754 ± 120 (mean ± s.d.) with monoclonal antibodies to *P. berghei* (P.b.), *P. knowlesi* (P.k.), *P. vivax* (P.v.) and *P. falciparum* (P.f.), respectively.

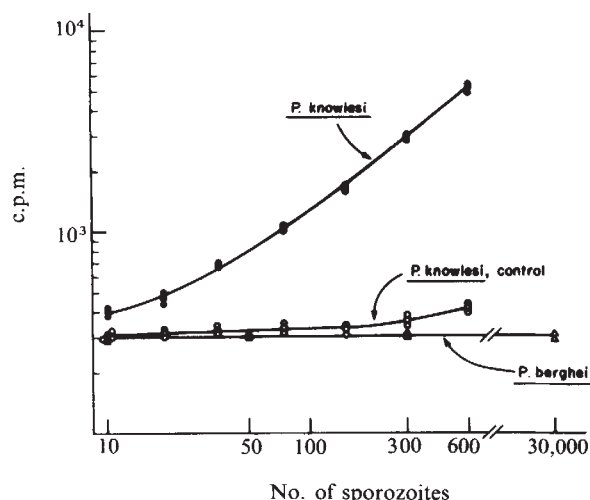


Fig. 2 Two-site immunoradiometric assay for the detection of *P. knowlesi* antigens. Salivary glands infected with *P. knowlesi* or *P. berghei* sporozoites obtained from *A. dirus* and *A. stephensi*, respectively, were placed in a 300- μ l solution of PBS containing 1% BSA and protease inhibitors antipain (25 μ g ml⁻¹), leupeptin (25 μ g ml⁻¹), aprotinin (2 trypsin-inhibiting units per ml) and phenylmethylsulphonyl fluoride (1 mM). The number of sporozoites in the suspension was counted under the microscope, and then the sample was sonicated (100 W for 3 min), centrifuged at 10,000g for 10 min, and the supernatant stored at -70°C. Microtitre plates of polyvinyl chloride were incubated overnight with 50 μ l of PBS containing a monoclonal antibody against *P. knowlesi* in a final concentration of 50 μ g ml⁻¹. After repeated washings with PBS-BSA, 35- μ l dilutions of antigen extracts from *P. knowlesi* or, as control, *P. berghei*, were placed in each well and incubated overnight at 4°C. After washing extensively with PBS-BSA, 30 μ l of a different ¹²⁵I-labelled monoclonal antibody against *P. knowlesi* were added. The plates were incubated at room temperature for 2 h, washed and the wells counted. ●, Anti-*P. knowlesi* in solid phase, plus *P. knowlesi* antigen; ○, anti-*P. berghei* in solid phase plus *P. knowlesi* antigen; △, anti-*P. knowlesi* in solid phase plus *P. berghei* antigen.

tional to the concentration of antigen in the extract⁵. In the example shown in Fig. 2, we detected less than 100 *P. knowlesi* sporozoites in a volume of 30 μ l, suggesting that this assay could be used to measure concentrations of parasites in extracts of pooled *Anopheles* from endemic areas. Indeed, we treated 12 pools of 25 mosquitoes as described in Fig. 2 legend, except that in this case the whole thoraxes and heads were extracted. Ten of these pools contained one mosquito from a population of *A. dirus* known to be 90% infected with *P. knowlesi*. The IRMA assay was then performed on all extracts. The number of counts in wells with extracts from pools which presumably contained an infected mosquito ranged from 1.6×10^4 to 2.5×10^4 , while the counts in the control wells were between 6×10^2 and 10^3 . In a separate set of controls containing extracts of single infected mosquitoes, the number of counts was not significantly different from those in the experimental set, indicating that the presence of large amounts of contaminating material from uninfected *Anopheles* did not interfere with the results.

We estimate that each infected mosquito in this batch contained $\sim 3 \times 10^4$ sporozoites. The sporozoite loads in mosquitoes from endemic areas have seldom been evaluated. In one region of Africa, Pringle⁶ found that the mean number of sporozoites in the salivary glands was 1.2×10^4 . It seems possible, therefore, that the two-site IRMA could be successfully used to measure the infection loads in batches of mosquitoes captured in endemic areas. This could be of epidemiological interest, as mosquitoes with heavier infections should be the more efficient vectors. Moreover, McGregor⁷ has suggested that the number of sporozoites inoculated at the moment of infection may determine the outcome of the disease.

The value of these two immunoassays in epidemiological studies remains to be established. They appear to possess the

sensitivity and simplicity which made them readily applicable, and 100% specificity is apparently achievable. The same methodology could be used to detect other human or animal parasites in crude extracts of vectors, provided that specific monoclonal antibodies are available.

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Murine model for pertussis vaccine encephalopathy: linkage to *H-2*

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Local, systemic and neurological complications have been observed following pertussis (whooping cough) vaccination in children^{1,2}. These often occur soon after primary or secondary immunization³⁻⁶. The neurological syndrome ranges from minor irritability to convulsions, coma, and on rare occasions death. Children who recover show varying degrees of permanent neurological sequelae. The pathogenesis of this encephalopathy is unknown, and the lack of an animal model has retarded our understanding of this disease. We describe here an animal model for pertussis immunization encephalopathy. A clinical syndrome with pathological features closely resembling post-immunization encephalopathy can be reproduced in the mouse after immunization with heat-killed *Bordetella pertussis* vaccine and exposure to a foreign antigen, if the inoculated animal has a susceptible *H-2* genotype.

During a study of the effects of *B. pertussis* as an adjuvant in experimental allergic encephalomyelitis (EAE)⁷, we discovered a lethal shock-like syndrome in BALB/c mice after immunization with heat-killed *B. pertussis* vaccine and sensitization to bovine serum albumin (BSA). Heat-killed *B. pertussis* (3×10^{10}) were injected into the tail vein of BALB/c mice on days 0 and 2. On days -1, +1 and +6, 1 mg BSA was given intraperitoneally (i.p.). Within 30 min to 2 h after injection of BSA, healthy mice became inactive and tachypneic. They developed myoclonic jerks of the trunk and extremities, exacerbated by tactile and auditory stimulation. They became moribund and most died within 2 h.

The requirements for expression of this disease are summarized in Table 1; both pertussis vaccine and at least 1 mg of BSA were necessary. Neither ovalbumin, diphtheria-tetanus toxoid (DT) vaccine, nor endotoxin could be substituted for BSA. We have also tested myelin basic protein, lysozyme, and an IgM phosphorylcholine binding myeloma, MOPC 104E,

Table 1 Requirements for induction of encephalopathy in BALB/c mice

Pertussis vaccine	Sensitizer			Endotoxin	DT vaccine	Result on day 6	
	BSA (1 mg)	BSA (<1 mg)	OA (1 mg)			No. dead	No. alive
+	+					31/35	4/35
+						0/10	10/10
+	+					0/5	5/5
+		+				0/5	5/5
+			+			0/5	5/5
+				+		0/10	10/10
+					+	0/15	15/15

Heat-killed *B. pertussis* 30 × 10⁹ (Massachusetts Department of Public Health Lots 258–259) were injected on days 0 and +2 into BALB/c mice (Radiobiology Colony, Stanford). Sensitizer, BSA, ovalbumin (OA; Sigma), 100 µg *Escherichia coli* LPS 055 (Difco)³², or DT vaccine (Wyeth), diluted 1:100, was given i.p. in 1 ml phosphate-buffered saline, on days –1, +1 and +6.

none of which promoted encephalopathy with pertussis vaccine. In contrast, various sources of BSA present in fetal and adult bovine sera produced encephalopathy together with pertussis vaccine. Animals that had received only the sensitizing antigen or *B. pertussis* vaccine alone did not develop the disease.

It is interesting that use of several inbred mouse strains suggested that susceptibility or resistance to pertussis immunization encephalopathy is associated with the major histocompatibility complex (*H-2*). Mice possessing *H-2^d* or *H-2^{s/d}* genotypes were highly susceptible (57/65 mice died; see Table 2), while mice of the *H-2^b* genotype were totally resistant (none of 44 mice died; Table 2) ($\chi^2 = 76.4$, $P < 0.0001$), and *H-2^k* mice demonstrated partial susceptibility (12/30 mice died; Table 2). Use of *H-2*-congenic inbred strains on a BALB/c background clearly supported this *H-2*-linkage. These strains are genetically identical except for differences at the *H-2* complex. BALB/c mice (*H-2^d*) are highly susceptible (31/35 died; Table 2), BALB.B mice (*H-2^b*) are totally resistant (0/15 died; Table 2), and BALB.K mice (*H-2^k*) are intermediate in sensitivity (7/15 died; Table 2).

Whether the genetic restriction in our system is imposed at the level of *B. pertussis* sensitivity or BSA sensitivity is unknown. BSA at low doses is under Ir gene control in the guinea pig⁸, and ovalbumin antibody responses are under genetic control in the mouse⁹. In mice the pertussis histamine-sensitizing factor segregates independently from *H-2* (ref. 10). In our pertussis immunization model we have found susceptibility genes outside *H-2*, as well as within it. During these studies we found one colony of BALB/c mice (Department of Medical Microbiology, Stanford University) that demonstrated reduced susceptibility to pertussis immunization encephalopathy (4/20 mice dead) compared with our other BALB/c colony (Department of Radiobiology, Stanford University) (31/35 dead; Table 2). Both BALB/c mouse colonies were shown to be unequivocally *H-2^d*. This was demonstrated by the ability of well defined anti-*H-2^d* monoclonal antibodies and alloantisera to selectively kill BALB/c spleen cells in microcytotoxicity assays^{11,12} (data not shown). Additionally, when ³⁵S-methionine-labelled spleen cell extracts from both BALB/c sources were immunoprecipitated with anti-*H-2^d* monoclonal antibodies or alloantisera and analysed by two-dimensional gel electrophoresis, we observed a pattern characteristic of the *H-2^d* haplotype^{13,14} (data not shown). Taken together, these results indicate that susceptibility to pertussis immunization encephalopathy is not under the control of *H-2* genes alone, but that factors outside *H-2* are involved also.

Post-mortem examination of the brain after immunization in susceptible BALB/c mice revealed diffuse vascular congestion and parenchymal haemorrhage in both the cortex and white matter. Cortical neurones showed ischaemic changes. Occasional areas of hypercellularity were evident in the meninges. Resistant BALB/b mouse brains revealed no pathological changes. The neuropathology in our mouse model resembles that of human cases in whom death occurred quickly after immunization^{15–20}.

B. pertussis has a wide range of physiological effects including increased IgE production²¹, increased sensitivity to anaphylactic shock²¹, lymphocytosis²², and hyperinsulinaemia²³. Its ability to induce increased vascular permeability may account for the tendency to produce haemorrhage. Pertussis may have potent effects particularly on brain vasculature, which may explain its usefulness as an adjuvant in hyperacute EAE¹². The time kinetics of this response suggest an anaphylactic reaction. In general, the central nervous system (CNS) is usually spared in anaphylaxis for most species²⁴. Whether *B. pertussis* can induce an anaphylactic CNS reaction in a susceptible animal must be considered. The role of IgE antibody in this system is now being investigated.

Perhaps the least apparent aspect of the murine model is the relevance to human disease of sensitization with BSA. Almost all babies exposed to cow's milk have serum antibody titres (IgG, IgA and IgM) to BSA^{25,26}. Even breast-fed babies have these serum antibodies, which are probably secondary to sensitization to BSA present in the mother's milk^{26,27}. Thus, human babies immunized against *B. pertussis* almost always have pre-existing anti-BSA antibody. This sensitization to BSA may lead to a similar chain of events following pertussis immunization in a genetically susceptible human.

The murine model described here predicts that genes acting at the level of the major histocompatibility complex may control susceptibility to *B. pertussis* immunization in humans. While susceptibility to many human diseases and to some allergic conditions has been linked to histocompatibility locus antigens in humans²⁸, the possibility that adverse reactions to routine immunization may be under genetic control seems novel. Immunization programmes are occasionally associated with neurological complications²⁹. The development of animal models to study the pathogenesis of immunization-induced neurological complications seems warranted. As pertussis immunization poses a major public health controversy in the

Table 2 *H-2*-linkage of pertussis immunization encephalopathy

	<i>H-2</i> type	Result on day 6	
		No. dead	No. alive
BALB/c	d	31/35	4/35
DBA/2	d	13/15	2/15
C3H.OH	d	3/5	2/5
(SJL/J × BALB/c) F ₁	s/d	10/10	0/10
BALB.B	b	0/15	15/15
B10	b	0/15	15/15
C3H.SW	b	0/14	14/14
SJL/J	s	5/15	10/15
A.SW	s	2/13	11/13
A.TH	s	0/15	15/15
BALB.K	k	7/15	8/15
C3H/DiSn	k	5/15	10/15

Mice were immunized with heat-killed *B. pertussis* (30 × 10⁹) on days 0 and +2, and 1 mg BSA i.p. on days –1, +1 and +6.

UK³⁰ and elsewhere³¹, this model system may provide insight into this critical problem.

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Cloned HBV DNA causes hepatitis in chimpanzees

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Most of our knowledge of the structure and function of the hepatitis B virus (HBV) genome comes from the analysis of the viral DNA sequences cloned in bacteria¹⁻³. Because the physical state of cloned HBV DNA differs from HBV DNA encapsidated in the virion—for example, it lacks the nick-gap structure⁴, and a covalently linked protein⁵—the question arises as to whether it can initiate HBV replication *in vitro*⁶ or *in vivo*. We describe here the development of typical acute viral hepatitis, and the detection of HBV-specific DNA sequences in the serum and liver, in a chimpanzee inoculated with cloned HBV DNA. This demonstrates that neither the virion proteins nor the nick-gap structure of the virion DNA are needed for the initiation of replication of HBV *in vivo*.

Table 1 Composition of the DNA mixture used for inoculation and transfection of chimpanzee

Physical state	HBV DNA species		
	HBV 14	HBV 2	HBV 6
Tandem duplications	pSHH 14.3 (40 pmol)	pSHH 2.1 (8 pmol)	
Closed rings	pHBV 14.1 (8 pmol)		pHBV 6 (6 pmol)

Source, physical state and amount of HBV-containing plasmids in the DNA mixture used for inoculation of the chimpanzees. pHBV 14.1 contains a full length DNA fragment of HBV-14 DNA inserted into the *Pst* site of pBR322¹⁰. pSHH 14.3 contains a tandem duplication of the same DNA fragment in the *Pst*I site of the vector plasmid pSV010¹¹. pSHH 2.1 contains HBV-2 DNA inserted as a tandem duplication into the *Eco*RI site of the vector plasmid pSV08¹¹. pHBV6 contains the whole HBV-6 genome as an *Eco*RI fragment inserted into the *Eco*RI site of pBR325¹². Closed HBV DNA circles were prepared by digestion of plasmids pHBV 14.1 and pHBV-6 with *Pst*I and *Eco*RI, respectively, and ligation with T4 ligase in conditions of preferential circularization of the HBV DNA fragments essentially as described⁶. The vector molecules were not removed from the DNA preparations as they do not replicate in animal cells, as holds true also for the pSV vectors that contain the origin of replication of SV40 but need large T antigen to replicate. The four DNAs were mixed in the ratio indicated, ethanol-precipitated and resuspended in 2 ml TNE (100 mM Tris pH 7.2, 155 mM NaCl, 0.1 mM EDTA). This DNA mixture was used for inoculation of the chimpanzee.

Three isolates of cloned HBV DNA derived from HBV of different serotypes were used in this study (Fig. 1), in an attempt to compensate by complementation or recombination for possible defects in the cloned DNA. The cloned HBV DNA was used in the form of closed circular and tandemly repeated molecules. A mixture of four of these DNA species (Table 1) was inoculated into a healthy chimpanzee intravenously, intramuscularly and directly into the liver. An autologous liver cell suspension transfected *in vitro* with the same DNA mixture was also inoculated into the liver. We adopted this approach of exposing the chimpanzee to a DNA mixture by different routes because no comparable experiments have previously been reported for HBV DNA, the chances for a successful outcome of such experiment seemed low, and also because of the limited number of available animals.

After inoculation, serum was regularly examined for liver function abnormalities, serological markers specific for HBV, and the presence of HBV DNA. Liver biopsies were examined histologically and for HBV DNA. Seven weeks after inoculation, the chimpanzee developed a typical, mild, self-limited, acute hepatitis. HBV surface antigen (HBsAg) subtype 'ay' (identified by subtype-specific antisera) appeared a week before an increase in the serum enzymes aspartate aminotransferase (AST) and alanine aminotransferase (ALT). About 1-13½ weeks later the first signs of the typical histology of a mild, acute hepatitis became recognizable in the liver biopsies (Table 2). On day 69, occasional portal mononuclear infiltration without obvious liver cell necrosis was seen. By day 81, a typical picture of acute viral hepatitis had developed. On day 101, resolving hepatitis was present. No further pathological changes were observed in a subsequent biopsy. HBV 'e' antigen (HBeAg) became detectable about 2 weeks after HBsAg, and both disappeared about 3 weeks later. The development of antibodies against HBsAg, HBeAg and HBV core antigen (HBcAg) and the kinetics of their appearance were normal. During the subsequent 9 months, there was no indication of the development of an HBV carrier state, or of chronic disease (serum bilirubin, serum enzymes and liver histology returned to and remained normal, and HBsAg and HBeAg did not persist), or of neoplastic liver disease (serum bilirubin, serum enzymes, α -fetoprotein and carcinoembryonic antigen levels remained normal).

A similar pattern of disease was recorded in a repeat experiment with a second chimpanzee. In contrast, no signs of disease were observed in the year following the administration, by intravenous injection alone, of the same HBV mixture to another chimpanzee.

To test whether the hepatitis was accompanied by replication of HBV DNA and the production of hepatitis B virus, DNA samples from blood and liver were analysed by the Southern technique⁷ with a ³²P-labelled, HBV-specific DNA probe. Sequences the size of viral DNA (3.2 kilobases (kb)) were present in the serum at the late, acute stage of the hepatitis (Fig. 2, lanes 1–3). Since the 3.2-kb band was much sharper after DNA repair synthesis the sequences were probably in the form of partially single-stranded virion DNA molecules. Cleavage with *Bgl*II yielded a major DNA species of 2.7 kb, as expected from HBV-14 or HBV-6 DNA circles but not from HBV2-DNA (see Fig. 1). Together with the result of the subtype determination (ay), this suggests that the major form of HBV DNA in the chimpanzee during hepatitis was HBV-14 DNA, the major species present in the mixture used for DNA transfer (Table 1).

The liver contained much more HBV DNA ($\sim 5 \times 10^8$ HBV DNA molecules per g of tissue) than serum ($\sim 10^6$ molecules ml^{-1}) and a more complex spectrum of HBV specific DNA molecules, which were predominantly non-integrated. They differed significantly from the DNA mixture used for transfection in the relative proportion of the different size molecules and in their restriction patterns (Fig. 2, lanes 7 to 12), indicating that a selective replication and possibly some rearrangement of the inoculated species of DNA had occurred. *Eco*RI and *Pst*I digestion both yielded a major 3.2-kb band (Fig. 2, lanes 9, 11), showing the presence of at least two of the inoculated DNA species (see Fig. 1). *Bgl*II produced a 2.3-kb DNA fragment, but not the 2.7-kb fragment produced from serum DNA (Fig. 2, lanes 3, 5), suggesting that HBV-2 was the predominant DNA species in the liver, but not the serum. Complex results were also obtained with other restriction endonucleases (data not shown), and we cannot draw any definite conclusion yet about the relationship between the several DNA species in the liver and the serum.

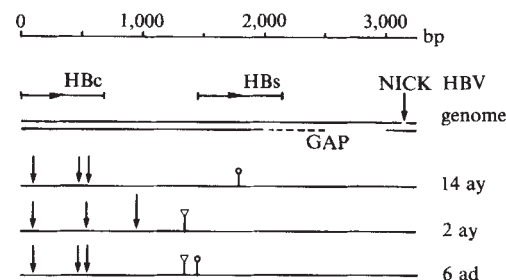


Fig. 1 Features of the HBV genome and cloned HBV species used for transfection. The circular HBV genome has been opened at nucleotide 1 of the HBc gene. Positions and orientations of the genes for HBsAg and HBcAg are indicated on top using the numbering system of Pask et al.². For characterization of the HBV genomes used for transfection, three key enzyme cleavage maps are indicated ($\downarrow$ *Bgl*II, $\uparrow$ *Pst*I, ∇ *Eco*RI). The serotypes of the cloned HBV genomes as determined for the HBsAg produced in tissue culture cells, using SV40 expression vectors containing defined HBV DNA fragments, are also presented (H.W., unpublished). The three DNA species originate from independent clonings of HBV DNA. HBV-14 DNA was isolated from the HBV bank of Burrell et al.¹⁷ by N. Gough. A restriction map of this clone has been described by N. Gough and K. Murray²¹ and its complete HBV sequence has been determined²². HBV-2 DNA was originally isolated as a phage λ clone by Charnay et al.¹⁸ and donated in plasmid pTKH10 by P. Tiollais. HBV-6 DNA originally cloned in pAO1¹⁹ was a gift of J. Summers.

That it was possible to induce the viral disease with all its usual clinical characteristics by inoculating cloned HBV DNA indicates that initiation of HBV replication requires—at least in these experimental conditions—neither the nick gap structure of the HBV genome as present in the virion (Dane particle) nor the virion proteins, including the viral DNA polymerase. That the hepatitis induced by cloned DNA followed a normal course is important because transfection of liver cells with DNA could have led to a different form of host cell–virus interaction. We are now trying to establish if the disease only develops after inoculation directly into the animal's liver, with or without

Table 2 Course of hepatitis B after transfection with cloned HBV DNA

Days after infection	AST units	ALT units	HBsAg ng ml ⁻¹	Anti-HBs mIU	Laboratory determinations				Histology	DNA in:	
					Anti-HBc titre	Anti-HBc IgM titre	HBsAg titre	Anti-HBe titre		Serum	Liver
-52	23	36	<0.1	<1	—	ND	—	—	ND	ND	ND
0	21	33	<0.1	<1	—	ND	—	—	ND	ND	ND
11	18	41	<0.1	<1	—	ND	—	—	ND	ND	ND
18	15	15	<0.1	<1	—	ND	—	—	ND	ND	ND
25	15	55	<0.1	<1	—	ND	—	—	ND	ND	ND
32	18	23	<0.1	<1	—	ND	—	—	ND	ND	ND
39	20	15	<0.1	<1	—	ND	—	—	ND	ND	ND
46	21	25	<0.6	<1	—	ND	—	—	ND	ND	ND
53	60	80	5	<1	—	<100	—	—	ND	ND	ND
56	22	75	11	<1	—	<100	—	—	—	ND	+
62	14	30	182	<1	—	<100	43	—	—	ND	+
69	ND	ND	470	<1	1	<100	80	—	(+)	—	+
76	77	194	<0.1	5	75	1,000	—	+	+	+	+
95	15	43	<0.1	6	100	1,000	—	+	ND	ND	ND
101	ND	ND	<0.1	7	70	<100	—	+	(+)	ND	ND
109	18	23	<0.1	11	45	<100	—	+	ND	ND	ND
116	11	21	<0.1	22	380	<100	—	+	ND	ND	ND
179	15	33	<0.1	141	400	<100	—	+	—	ND	ND

Course of disease in a chimpanzee infected with cloned DNA. A colony-born, 3-yr old female was kept in specially designed isolation facilities. On day 0, 1 mg atropine and 160 mg ketamine hydrochloride were given, and anaesthesia was maintained with an oxygen–nitrous oxide–fluothane mixture. A surgical liver biopsy was taken. 500 μ l of each of the mixture of HBV DNAs described in Table 1 were inoculated intravenously and intramuscularly, and 900 μ l directly into the liver. In addition, 900 μ l of a liver cell suspension transfected previously *in vitro* with 100 μ l of the same DNA mixture was inoculated into the liver. Transfection was carried out using DEAE dextran (molecular weight 2×10^6 ; Sigma) as a facilitator in a modification of the method described previously^{13,14}. Liver biopsies (cylinders of liver tissue with a diameter of 1–2 mm, and a length of 9–15 mm) were taken from chimpanzees using Menghini needles, and transferred immediately into Williams medium (Flow Laboratories) containing 100 mM Tris-HCl pH 7.2 and 0.1 mM EDTA (WD-medium). The liver biopsies were washed three times with WD-medium and the cell suspension was prepared by mechanical disruption of the liver tissue. The liver cells were resuspended in 10 ml WD-medium, centrifuged for 5 min at 1,000 r.p.m. at room temperature, resuspended in 10 ml Williams medium (Flow Laboratories), recentrifuged for 5 min at 1,000 r.p.m. at room temperature and resuspended in 800 μ l TNE buffer containing 100 μ l $10 \times$ RPMI-1640 medium (Flow Laboratories). To this, 100 μ l of the DNA mixture and 500 μ g DEAE dextran dissolved in 100 μ l TNE were added. The mixture was incubated for 1 h at 37°C and reinjected into three different sites of the animal's liver under visual observation. All subsequent surgical or Menghini needle liver biopsies and bleedings were performed under general anaesthesia. AST, ALT, bilirubin and γ -glutamyl transpeptidase (γ -GT) were determined by standard techniques. Normal chimpanzee AST values: ≤ 40 units l^{-1} ; normal chimpanzee ALT values: ≤ 40 units l^{-1} . Bilirubin and γ -GT levels did not exceed normal values (≤ 2 μ M total bilirubin, and ≤ 35 units l^{-1} γ -GT) throughout the study. HBsAg, anti-HBs, anti-HBc, anti-HBV IgM, HBeAg and anti-HBe were determined by commercially available (Abbott), or self-prepared^{15,16} enzyme or radioimmunoassays. HBsAg was measured in a radioimmunoassay calibrated with a standard preparation. HBsAg subtypes were determined in the serum obtained 69 days after inoculation by blocking radioimmunoassays with monospecific 'ad' or 'ay' antibodies. Anti-HBs, anti-HBc and anti-HBc IgM were titrated as described previously¹⁵. HBeAg was titrated in a radioimmunoassay, and the reciprocal of the last serum dilution with a ratio of more than 2.1 of sample c.p.m.:negative control c.p.m. is given. Anti-HBe is indicated only as positive or negative. Serum (S) and liver (L) were tested for DNA; + or —, sample positive or negative for HBV DNA. Detailed results are given in the text and in Fig. 2 legend. ND, not determined.

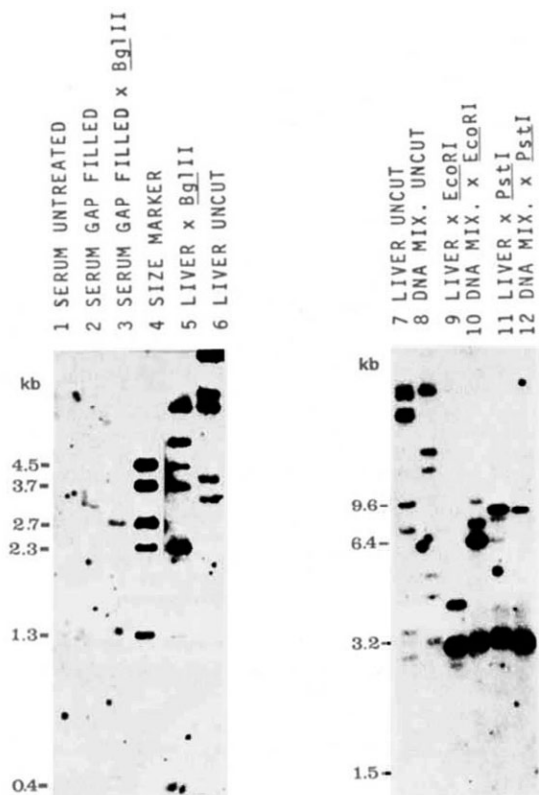


Fig. 2 Southern blot analysis of HBV DNA present in chimpanzee serum and liver. Serum taken 76 days after inoculation was clarified by centrifugation for 20 min at 4°C and 15,000 r.p.m. in a SW40 spinco rotor. The supernatant was recentrifuged for 3 h at 30,000 r.p.m. and the virus pellet sedimented through a 5–30% sucrose gradient at 35,000 r.p.m. for 12 h. DNA was isolated from the virus pellet using a proteinase K/SDS/phenol procedure^{19,20}, and part of the DNA was gap-filled with *Escherichia coli* DNA polymerase I. A piece of liver tissue from a surgical biopsy taken 69 days after transfection was minced, homogenized in TNE buffer and the DNA extracted as described above. For blot analysis samples corresponding to 1 ml serum or 20 µg liver DNA were loaded on two agarose gels before and after cleavage with restriction endonucleases. For the *Bgl*II analysis (lanes 3, 5) a *Bgl*II cleaved mixture of pSHH 14.3 and pSHH 2.1 was used as a reference in lane 4. The size marker for the second gel is not shown. Both gels were blotted onto nitrocellulose and hybridized with ³²P-labelled HBV DNA isolated from pSHH 2.1 (specific activity 10⁸ c.p.m. per µg of DNA)⁷. Lanes 1–3 were exposed for 3 weeks, other lanes for shorter times.

transfection of liver cells *in vitro*, or whether it could be produced by intravenous inoculation. These experiments will provide more data about the possible risk of recombinant DNA molecules from animal viruses, and are expected to extend to a human pathogen those conclusions drawn previously from the polyoma/mouse model system^{8,9}.

The experimental system described here will also allow the identification *in vivo* of infectious HBV DNA molecules for development of cell or tissue culture systems for HBV replication. Meanwhile, this system will enable us to study *in vivo* the genetics of HBV using recombinant plasmids constructed *in vitro*, and to test different parts of the HBV DNA for their role in virus replication, their effect on host cells, and their contribution to the possible oncogenicity of the HBV genome.

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Rearrangements between the immunoglobulin heavy chain gene J_H and C_μ regions accompany normal B lymphocyte differentiation *in vitro*

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Before B cells can synthesize their first immunoglobulin (Ig) molecules, membrane IgM, one of the many V regions, a D region, and a J region adjacent to the single C_μ region, must come together with the deletion of the intervening DNA^{1–9}. Isotype switching during B-cell development is accompanied by reassociation of the fused VDJ complex with a C_H region downstream from C_μ . The exact points of reassociation are called switch sites, and have been located 5' to each C_H gene^{10–12}. IgM-bearing B cells isolated from murine spleen showed no rearrangements in the area of switch sites between J_H and C_μ ^{7–9}. We now present results showing that the maturation of these cells to plasmablasts displaying isotype switching and immunoglobulin secretion in cultures containing bacterial lipopolysaccharide (LPS) is accompanied by DNA rearrangements between J_H and C_μ sequences. As these plasmablasts arise in cultures of LPS stimulated cells that undergo few divisions, display IgM to IgG isotype switching, and evolve into secretory cells, it is suggested that these rearrangements may have a role in normal B-cell development.

Rearrangements between J_H and C_μ sequences were first detected among immunoglobulin-secreting hybridoma and plasmacytoma populations^{5–7}. However, these rearrangements were largely dismissed due to the possibility that they represented tumour artefacts in these cell populations⁹. We therefore sought a system in which normal plasmablasts could be examined, and chose to analyse B lymphocytes after stimulation in culture with LPS¹³. An ethanol fixation technique allowed penetration of fluorochrome labelled anti-IgM into the cells without causing DNA damage or cell aggregation. Using this procedure, cells could be analysed and sorted according to their secretory IgM content with a fluorescence activated cell sorter (FACS). Figure 1a shows a size distribution of cells stimulated *in vitro* for 4 days with LPS, and stained for cytoplasmic IgM. Figure 1b shows the fluorescence distribution of the sized population indicated by arrows in Fig. 1a. The plot indicates that 20% of the cells are negative, while 80% compose a distinct fluorescent population showing staining with the anti-IgM reagent.

The negative population was not apparent on analysis of similar cultures treated with anti-Thy 1.2 plus complement to remove T cells and then recultured overnight to remove adherent and dead cells before fixation and staining (Fig. 1c). This finding suggests that most LPS-stimulated B cells develop at

least a small amount of IgM in their cytoplasm. Cells that show large amounts of cytoplasmic fluorescence after staining are perhaps those that have reached an active stage of IgM secretion. The 15–20% of LPS-stimulated cells that have switched from expression of IgM to IgG isotypes¹³, which we have detected by fluorescence microscopy, may retain only residual amounts of IgM and therefore contribute to the population showing only faint fluorescence in the FACS. To analyse the C_{μ} region context of cells that have been effectively producing cytoplasmic IgM, and likely secreting it, the 30% most fluorescent (30%+) cells were collected. The 30% of the cells with the least fluorescence were also isolated in the hope of enriching for cells having switched surface isotype (30%–, Fig. 1d). Cell populations ($1-5 \times 10^6$) were then lysed to prepare DNA, and Southern blot analysis was used to compare the context of C_{μ} regions in the two populations of LPS-stimulated plasmablasts with that of the C_{μ} regions from mouse embryos.

Figure 2a depicts the C_{μ} locus, the map of cleavage sites for restriction enzymes, and the C_{μ} probe used in Southern blot analysis. The map shows a *Bam*HI restriction site within the germ-line C_{μ} gene and close to the 5' side. Digestion of embryonic DNA with this enzyme, Southern blotting, and hybridization with the ³²P-labelled, cloned C_{μ} probe¹⁴ (see Fig. 2 legend) reveals major (3' fragment), and minor (5' fragment) bands of approximately 12 kilobases (kb) and 10 kb respectively (Fig. 2b, lane 4). The band intensity associated with the 3' C_{μ} fragment may be used to approximate the content of C_{μ} regions in DNA from lymphoid populations, as this band position would not alter following rearrangements 5' to the C_{μ} region. In contrast, bands representing either the embryonic 5' *Bam*HI fragment (Fig. 2b, lane 4) or the *Eco*RI digestion fragment (Fig. 2c, lane 4) would each be susceptible to size alteration following J_H-C_{μ} rearrangements.

Figure 2 shows DNA digests from 30%+ (Fig. 2b and c, lane 5) and 30%– (Fig. 2b and c, lane 6) populations digested with either *Bam*HI (Fig. 2b) or *Eco*RI (Fig. 2c) restriction enzymes, and compared by Southern blot analysis to 2, 4, 6 and 8 μ g of embryonic DNA (Fig. 2b and c, lanes 1–4). In the 30%+ cellular DNA, the *Bam*HI 3' fragment compares most closely in density to that of 6 μ g of embryonic DNA. However, the 5' *Bam*HI and the *Eco*RI fragments are each clearly less intense than the corresponding bands representing 6 μ g of embryonic DNA (compare Fig. 2b and c, lane 5 with lane 3). In the 30%– cellular DNA, the *Bam*HI 3' band compares most closely in intensity to that of 4 μ g of embryonic DNA. Again, the 5' *Bam*HI and the *Eco*RI fragments are clearly less intense than the corresponding bands of 4 μ g of embryonic DNA (compare Fig. 2b and c, lane 6 with lane 2). These observations suggest that in both the 30%+ and the 30%– populations, C_{μ} genes remaining in an embryonic context on their 3' sides were heterogeneously rearranged on their 5' sides. The decreased intensity of the *Eco*RI band specifically localized most of these rearrangements to the region between J_H and C_{μ} sequences. Although the proportions of rearranged C_{μ} genes varied among cells cultured in LPS, at least 25% of C_{μ} genes were rearranged, as judged by changes between J_H and C_{μ} for both the 30%+ and 30%– populations in each of three experiments.

The recognition that rearrangements of C_{μ} regions commonly occur in the area of switch sites in the DNA of normal plasmablasts with both high and low cytoplasmic IgM contents, but not among the B cells from which they were derived⁷⁻⁹, suggests that this event may accompany normal maturation of B cells to secretory plasma cells and/or the switching of heavy chain isotype. Possible functions of these rearrangements are:

(1) A rearrangement or deletion between J_H and C_{μ} regions may affect transcription of the C_{μ} section of the *VDJ* C_{μ} complex, and/or processing of the primary transcript to favour increased production of secreted, rather than membrane, IgM. It has been reported¹⁵ that some plasmacytoma cells are capable of producing both forms of IgM by varying the processing of the same μ chain transcript. Thus, rearrangement to the 5' side

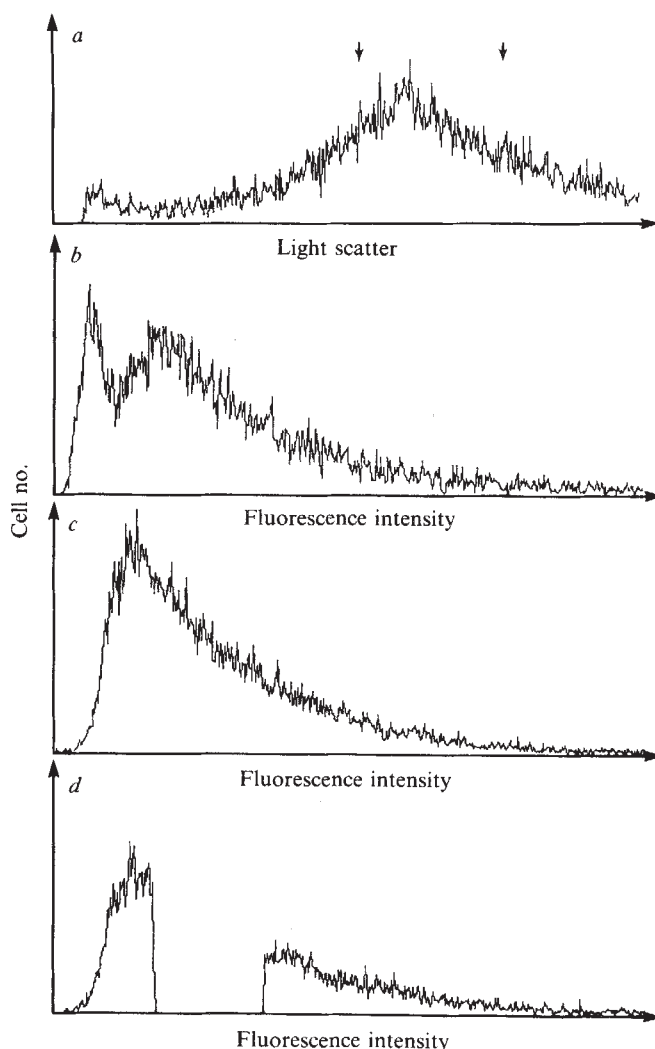
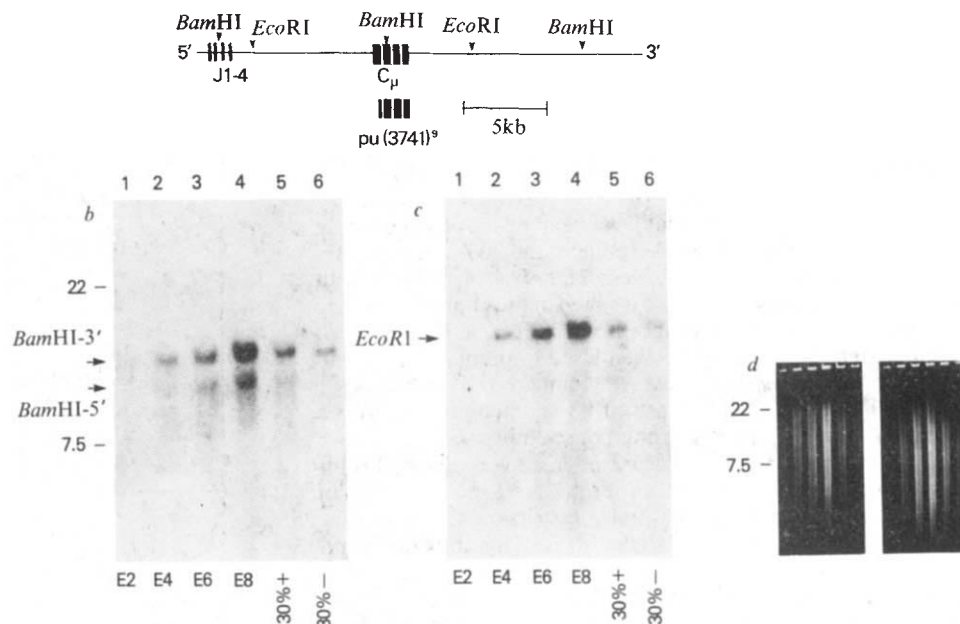


Fig. 1 FACS light scattering and fluorescence profiles of LPS-stimulated lymphocytes. BALB/c spleen cells were suspended in Hanks' balanced salt solution and washed once before resuspension in 20% fetal calf serum (FCS) in RPMI-1640, 50 μ g ml⁻¹ gentamycin, 4 mM glutamine, 5×10^{-5} M 2-mercaptoethanol, and 50 μ g ml⁻¹ LPS (Sigma, phenol extract from *Salmonella typhimurium*). Cells were cultured at 37°C, 5% CO₂ in air, at 10^6 cells per ml in 12-well Falcon culture plates as previously described¹³ for 4 days. Spleen cells were then collected from culture and passed through a cotton column. After centrifugation at 1,200 r.p.m. for 10 min, cells were resuspended in 5–20 ml culture medium. Absolute ethanol was added dropwise with agitation until a 90% solution was achieved. After 30 min on ice, cells were sedimented for similar dropwise readjustment and washing in Hanks plus 5% FCS. Pelleted cells were stained in suspension on ice with a fluorescein conjugated anti-IgM reagent, anti-CBPC 112¹⁷. The ethanol fixation in suspension rendered the plasma membrane permeable, allowing labelled antibody stain to reach the cell interior such that cytoplasmic (versus membrane) IgM could be detected. After a 30-min staining period, cells were washed in Hanks solution without phenol red with 5% FCS, and applied to a Beckton-Dickinson FACS II for sorting. **a**, Scatter profile of stained cells. The y-axis represents cell number in arbitrary units. The x-axis (also in arbitrary units) represents cell scatter. Cells of a size represented by points between the arrows were selected for further fluorescence analysis and sorting of plasmablasts. **b**, Fluorescence profile of anti-IgM stained plasmablasts. Cells of size indicated in **a** were analysed for fluorescence intensity. The x-axis represents fluorescence intensity; the y-axis represents cell number. **c**, Fluorescence profile of 4-day LPS cultured cells treated with anti-Thy 1.2 to remove T cells and plated overnight before staining to further remove adherent cells. For this procedure, an anti-Thy 1.2 containing supernatant from an *in vitro* culture of a rat monoclonal cell line (J1j) obtained from J. Sprent¹⁸ was diluted 1:10 in Hanks, to which cells were added at 2×10^7 cells ml⁻¹. After 30 min at 4°C, the cells were washed once and resuspended in guinea pig complement diluted 1:10. They were then incubated at 37°C for 45 min. Cells were passed over a cotton column, washed once and resuspended in Hanks plus 20% FCS. Cells which were first cultured for 4 days in LPS, followed by anti-Thy 1.2 treatment described here, were then plated on 24-well Falcon tissue culture dishes for 12 h at 4°C before staining and FACS analysis. **d**, Sorting profile. The 30% of the cells with the most fluorescence (right hand peak) and the 30% of the cells with the least fluorescence (left hand peak) were selected for DNA analysis.

Fig. 2 C_{μ} gene rearrangements in LPS-stimulated plasmablasts. **a**, Restriction enzyme map of the C_{μ} gene and the J_H region showing *EcoRI* and *BamHI* sites. DNA preparations were digested with either *BamHI* (**b**) or *EcoRI* (**c**) and electrophoresed through agarose, transferred to nitrocellulose and hybridized^{7,19,20} with the ³²P-labelled recombinant probe p_{μ} 3741⁹ described previously¹⁴. Lanes 1–4 (**b**, **c**) represent 2, 4, 6 and 8 μ g of embryonic DNA respectively. 12 μ g of sorted cell DNA (as estimated by A_{260} readings) was split evenly between *BamHI* and *EcoRI* digests. Lane 5 (**b**, **c**) contains 30% + DNA. 30% – DNA appears in **b** and **c**, lane 6. Final autoradiographs were scanned by densitometry to compare band intensities. **d**, The ethidium bromide stained gel before Southern blotting and hybridization. The figures alongside the gel indicate the approximate mobility of *EcoRI*-digested phage λ fragments expressed in kilobases.



of the C_{μ} gene may not be essential for expression of secreted IgM but may potentiate its production.

(2) The deletion or rearrangement of switch sites may affect the switching process either quantitatively or qualitatively. Extensive deletion in this area may preclude switching and lock the cell into IgM expression.

(3) Some rearrangements of C_{μ} regions detected during the B-cell development stimulated by LPS may be a consequence, rather than a precursor event, of the switching process. For instance, if sister chromatid exchange is involved in the switching process^{5,16}, the J_H - C_{μ} rearrangement may reflect the deletion of a C_{μ} gene from one chromatid and its passage to a non-embryonic configuration into the sister chromatid. Alternatively, if switching occurs by active deletion of intervening sequences, then excised C_{μ} genes would probably not be detected on Southern blots, unless they were of sufficient size and stability to be isolated in the preparation of chromosomal DNA.

Evaluation of possible roles for J_H - C_{μ} rearrangements in normal B cell development may be achieved by further analysis of DNA from cells after short term culture in LPS, as they represent normal plasmablasts that are actively secreting immunoglobulin and switching isotypes.

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Transforming gene of a human leukaemia cell is unrelated to the expressed tumour virus related gene of the cell

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The virally encoded oncogenes (*v-onc*) of avian and mammalian retroviruses are the recombinant products of normal cellular genes (*c-onc*) and a retroviral genome^{1,2}. These cellular homologues have been highly conserved during evolution and are found in human DNA^{1,2}. The expression of at least one *c-onc* under the control of a viral promoter results in transformation of cells in a manner resembling that displayed by the *v-onc* counterpart³; the inappropriate expression of *c-onc* in the absence of viral influences could likewise result in the malignant state⁴. This proposal would be strongly supported by the presence of *c-onc* RNAs in a variety of human tumours were they not also demonstrable in normal tissues^{5–7}. The role of these RNAs in the oncogenic process remains unclear. Here we report that RNA homologous to the oncogene (*v-abl*) of Abelson murine leukaemia virus (A-MLV) is expressed in a unique human leukaemia in a fashion different from that of other human tissues and tumours. In addition, DNA from this tumour transforms NIH-3T3 cells at a high efficiency in a transfection assay. The transfected sequences are not related to the *v-abl* gene, but the NIH-3T3 transformants manufacture a transforming growth factor which behaves similarly to factors produced by A-MLV-transformed NIH-3T3 fibroblasts.

The human leukaemia cell line, SMS-SB, was derived from the leukaemic lymphoblasts of a patient with an unusual pre-B acute lymphoblastic leukaemia⁸. SMS-SB closely resembles the majority of A-MLV-induced mouse leukaemias in its clinical presentation and in the phenotype of the tumour cells^{8–10}. The human and the mouse tumour both present as paravertebral masses with foci in the bone marrow and no thymic involvement. A-MLV-transformed lymphoid cells and SMS-SB cells display pre-B-cell surface markers, express cytoplasmic mu heavy

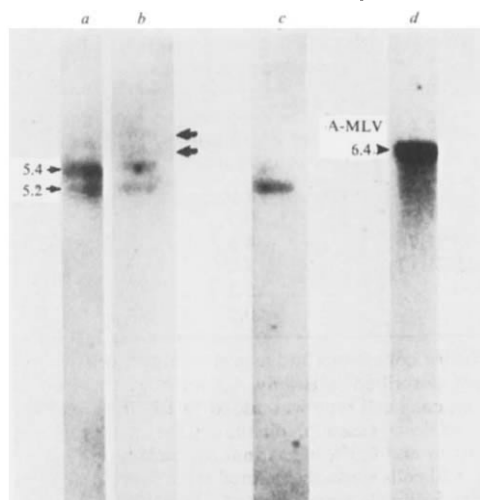


Fig. 1 Detection of unique species of *abl*-related RNA in the human leukaemia cell line, SMS-SB. Cytoplasmic RNA was extracted from tumour cell lines by the SDS-phenol method as modified by Wheeler *et al.*²⁵. Poly(A) RNA was purified by one passage over a poly(U)-Sephadex column²⁵. *abl*-Related sequences in poly(A)-selected RNAs were compared by Northern blot analysis. RNAs were denatured with glyoxal and fractionated by gel electrophoresis²⁶, blotted to nitrocellulose²⁷ and hybridized to ³²P-labelled pABSub9. pABSub9 (the gift of Drs J. Wang and D. Baltimore) includes essentially the entire *v-abl* region from A-MLV in pBR322²⁸. Hybridization and wash conditions followed the method of Thomas²⁷. Lanes *a* and *b* are two independent poly(A) RNA preparations from SMS-SB cells. Lane *c* is a poly(A) preparation from another human leukaemia cell line, NALL-1. Lane *d* is total cytoplasmic RNA prepared from a C57BL/6 mouse tumour induced by the A-MLV isolate that encodes a 160,000 molecular weight transforming protein²⁴. Arrows indicate species of RNA seen only in SMS-SB preparations.

chain molecules and demonstrate low levels of terminal deoxynucleotidyl transferase.

Using *v-abl*-specific nucleic acid probes in the Northern blot procedure, we have analysed the expression of *c-abl* (human) sequences in the SMS-SB tumour and in other human cells (Fig. 1). Two species of *abl*-related RNA, 5.2 and 5.4 kilobases (kb), are detected in the poly(A)-containing RNA from all human cells that we have examined, including a non-B, non-T acute lymphoblastic leukaemia cell line (NALL-1) (Fig. 1, lane *c*), another lymphoblastic leukaemia cell line (NALM-6), the leukaemic cells of a T-cell leukaemia patient, tonsil lymphocytes, phytohaemagglutinin-stimulated normal blood lymphocytes, Epstein-Barr virus-transformed lymphoblastoid cell lines, Simian virus 40-transformed human fibroblasts and an epidermoid carcinoma cell line, A431 (data not shown). In each case the 5.2-kb species was the most abundant. SMS-SB RNA is unique in that it contains four species of *abl*-related RNA, 5.2, 5.4, 6.2 and 6.8 kb, and the 5.4-kb species is always most abundant (Fig. 1, lanes *a*, *b*). The two high molecular weight RNAs have never been seen in other human cells. We have never detected *abl*-related RNAs of <5.2 kb in either tumour or normal cells as reported by others^{5,7}.

The unique expression of *abl*-related RNAs in SMS-SB cells is not due to gross rearrangements or duplications of genomic *c-abl* (human) sequences. This was determined by comparing the restriction enzyme pattern of *c-abl* (human) in SMS-SB with the pattern in genomic DNA from other human sources (Fig. 2, lanes *a*, *b*). However, *c-abl* (human) sequences are hypomethylated in SMS-SB DNA when compared with homologous sequences in another human leukaemia, a T-cell lymphoma, SMS-OJ (Fig. 2, lanes *c*, *d*). DNA which is hypomethylated at cytosine residues has been associated with enhanced transcriptional activity¹¹. When SMS-OJ DNA is digested with the methylation-sensitive enzyme *HpaII*, all *c-abl* (human) sequences remain in high molecular weight DNA with no discrete bands generated (Fig. 2, lane *d*). When SMS-SB

DNA is digested with *HpaII*, three discrete bands appear at 12, 18 and 20 kb (Fig. 2, lane *c*). This could suggest unique transcriptional signals in SMS-SB *c-abl* DNA not present in at least one other human leukaemia.

To test for the presence of an oncogene in SMS-SB DNA, high molecular weight DNA from SMS-SB cells was transfected into NIH-3T3 mouse fibroblasts which were observed for the development of transformed foci¹². This technique has been used to identify other purported human oncogenes¹³⁻¹⁷. SMS-SB DNA transformed NIH-3T3 cells at a high efficiency (1 focus per μ g DNA) and the resulting foci were picked and examined by Southern blots for the presence of *c-abl* (human)-related sequences (Fig. 3). DNA from each clone hybridized with a human probe, specific for repetitive primate DNA (*Alu* sequences)^{18,19}, indicating the presence of human sequences (Fig. 3, lane *f'*). However, when DNAs from the transformants are cleaved with the restriction enzymes *EcoRI* or *HindIII*,

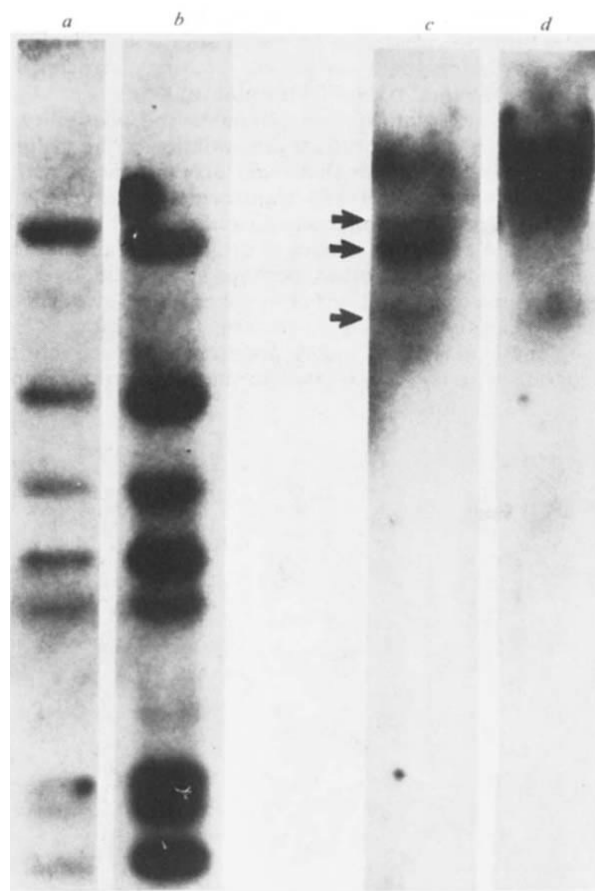


Fig. 2 Arrangement of *abl*-related sequences in DNA from human leukaemia cells. The arrangement of *c-abl* (human) sequences was analysed in DNA prepared from the SMS-SB tumour cell line (lanes *a*, *c*) and leukaemic lymphocytes from a patient with a T-cell lymphoma, SMS-OJ (lanes *b*, *d*). The DNAs were extracted, digested with restriction endonucleases, electrophoresed in agarose gels and blotted to nitrocellulose filters, all as previously described²⁹. The filters were hybridized to ³²P-labelled pABSub9 in 6 $\times$ SSC, 5 $\times$ Denhardt's, 0.5% SDS at 68 $^{\circ}$ C for 24-36 h. Lanes *a* and *b* compare the *c-abl* (human) *SacI* pattern in SMS-SB DNA (lane *a*) and SMS-OJ DNA (lane *b*). The variance in intensity is due to a twofold excess of SMS-OJ DNA. In lanes *c* and *d*, respectively, SMS-SB and SMS-OJ were digested with *HpaII*, a methylation-sensitive enzyme which recognizes the sequence CCGG but only cleaves the sequence if the second cytosine is not methylated. One can compare the relative methylation of a particular gene from two different DNA sources by the size of *HpaII* bands generated in a digest. When SMS-SB and SMS-OJ DNA are cleaved with *MspI*, an isoschizomer of *HpaII* which cuts at CCGG regardless of the methylation status of the second cytosine, an identical pattern is obtained in both cases (data not shown).

which produce distinctive *c-abl* (mouse) (lanes *b, e*) and *c-abl* (human) (lanes *a, d*) patterns, no human-specific fragments are revealed after hybridization to *v-abl* probes (lanes *c, f*). This result suggests that the *c-abl* (human) gene is not responsible for NIH-3T3 cell transformation.

The nature of the SMS-SB gene responsible for transformation of NIH-3T3 fibroblasts is suggested by the following experiments. A-MLV-transformed NIH-3T3 cells no longer bind ^{125}I -labelled epidermal growth factor (EGF)²⁰ and produce a transforming growth factor (TGF) which interacts with the EGF receptor and renders normal fibroblastoid cells anchorage independent²¹. We have found that both SMS-SB cells and the SMS-SB NIH-3T3 transformants also produce TGFs. Serum-free conditioned media from SMS-SB cells and all transformants tested thus far produce factors which render Rat-1 cells anchorage-independent while similar media from nontransfected NIH-3T3 cells do not induce growth of the cells in agar (Table 1). Further, the transformants do not bind ^{125}I -EGF (Table 1) and are responsive to dexamethasone in serum-free growth experiments (data not shown), another characteristic of A-MLV-transformed NIH-3T3 fibroblasts²¹.

The transfection data do not eliminate the possibility that *c-abl* expression was required for the initiation of the malignant process in SMS-SB cells or that *c-abl* acts in concert with the gene responsible for NIH-3T3 transformation, a gene which could induce growth factor production. Presumably, the SMS-SB cells are the end point of tumour progression, and may have reached a state in which other, perhaps synergistic, oncogenes are also activated. Several lines of experimental evidence support such a notion. Avian leukosis virus (ALV) induces B-cell lineage leukaemias in chickens apparently by activating an endogenous gene (*c-myc*) related to the transforming gene of

Table 1 NIH-3T3 (SMS-SB) transformants produce transforming growth factors (TGFs)

Source of TGF	% Colonies of Rat-1 cells in agar	Specific binding of ^{125}I -EGF (c.p.m. per 10^6 cells)
EGF	11.0	NT
NIH-3T3	0.3	9,400
NIH (SMS-SB9)	4.6	220
NIH (SMS-SB12)	7.0	120
SMS-SB	15.2	NT
No TGF	<0.2	NT

Growth factor collections and assays on Rat-1 cells were performed as previously described³¹. Briefly, 0.4 ml of serum-free conditioned medium from each cell type was added to 10^5 Rat-1 cells in 2.0 ml of Dulbecco's minimal essential medium (DMEM) supplemented with 10% calf serum and 0.3% agar (final concentration). (The number of colonies of >20 cells were determined after 3 weeks. For each experiment at least 500 cells were counted.) For the binding of ^{125}I -EGF, confluent 35-mm plates of each cell type were incubated in DMEM plus 1% bovine serum albumin at 37°C for 1 h with 20 ng ml⁻¹ ^{125}I -EGF (80,000 c.p.m. per ng). This is a saturating amount of EGF on the NIH-3T3 cells (data not shown). To determine specific binding, similar plates were incubated with 1 μg unlabelled EGF in addition to the ^{125}I -EGF. After binding, the number of cells per plate were determined.

another acutely transforming retrovirus MC29 (*v-myc*)²². However, when DNA from ALV-induced tumour cells is subjected to the NIH-3T3 transfection assay, fibroblast transformation is due to another gene, unrelated to the *c-myc* gene²³. In another case, when some murine A-MLV-induced tumours are transferred intraperitoneally in mice, they become more tumorigenic but lose the A-MLV genome²⁴. Apparently, another oncogene is activated in these cells. An analogous situation could exist in SMS-SB cells. SMS-SB cells may still retain a need for the *c-abl* gene product, or, alternatively, it may function to block the cells' further differentiation so that they remain susceptible to the activated oncogene. It has recently been shown that sequences related to the oncogene of another acute leukaemia virus, avian myeloblastosis virus, are differentially expressed in certain haematopoietic cell types⁶.

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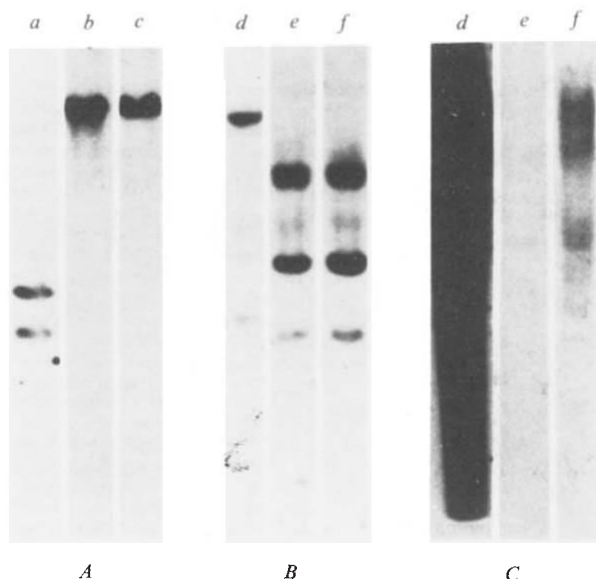


Fig. 3 Analysis of *abl*-related sequences in DNA from NIH-3T3 transformants. Transfections of high molecular weight DNA prepared from SMS-SB cells were performed by M.-A. Lane as previously described³⁰. Transformed foci [designated NIH(SMS-SB)] were selected, high molecular weight DNA was prepared, and the DNA was analysed by the Southern blot method as described earlier. The experiment shown above involves a single NIH (SMS-SB) transformant, NIH (SMS-SB12). Similar results were obtained with 11 other transformants. The normal *c-abl* (human) pattern is seen in lanes *a* and *d* (SMS-SB DNA). The normal *c-abl* (mouse) pattern is seen in lanes *b* and *e*. The pattern of *abl*-related sequences in NIH (SMS-SB12) is seen in lanes *c* and *f*. In panel *A*, the DNAs were cut with *EcoRI* and probed with pSA17, which is a molecular clone of *v-abl* sequences derived in our laboratory²⁹ and subcloned in the plasmid pSA801. It contains the 3' 60% of *v-abl* present in the p120-encoding A-MLV genome. In panel *B*, the DNAs were cut with *HindIII* and probed with pAB1sub9. In panel *C*, the filter used in panel *B* was hybridized to a human *Alu* probe, BLUR8.

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Chromosomal localization of human cellular homologues of two viral oncogenes

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Acute transforming RNA tumour viruses represent genetic recombinants between type C retroviral sequences and transformation-specific sequences of cellular origin. Of the known mammalian cell-derived transforming genes (oncogenes), two have been shown to encode proteins with either intrinsic or highly associated tyrosine-specific protein kinase activity. One such gene, *c-fes*, is of cat cellular origin, while the second, *c-abl*, was derived from mouse cellular sequences. We now show that the human equivalents of *c-fes* and *c-abl* are localized on human chromosomes 15 and 9, respectively. These findings exclude the possibility that these transformation-related genes are clustered at a single locus within the human genome. It is of interest that both of these chromosomes are involved in specific rearrangements found in certain forms of human cancer.

Two mammalian and four avian cell-derived viral oncogenes encoding proteins with tyrosine-specific protein kinase activity have been described¹. One of these, *c-fes*, is represented within the genomes of two independent isolates of feline sarcoma virus (FeSV), Gardner and Snyder–Theilen, both of which represent genetic recombinants between feline leukaemia virus and cellular (*c-fes*) sequences of cat origin^{2,3}. *c-fes* is closely related to *c-fps*, a transforming gene common to several independent avian transforming retrovirus isolates^{4,5}. The second cell-derived viral oncogene of this class, *v-abl*, has so far been identified as a transforming component only of the Abelson strain of murine leukaemia virus (MuLV). This virus is a genetic recombinant between Moloney MuLV and mouse cellular (*c-abl*) sequences⁶. The primary translational products of these two cell-derived viral oncogenes are polypeptides containing amino-terminal structural proteins encoded by the *gag* gene, covalently linked to acquired cellular sequence-encoded components^{7–10}. Tyrosine-specific protein kinase activities associated with such polypeptides^{11–15} have been implicated in transformation through the analysis of transformation-defective viral mutants^{16–18}. Recently, transforming sequences isolated from a human bladder carcinoma cell line have been shown to exhibit homology with acquired transforming (oncogenic) sequences of the Harvey strain of murine sarcoma virus^{19,20}. Because of the potential involvement of the cellular homologues of viral oncogenes in human cancer, we decided to investigate the human genetics of these sequences and to test the possibility that chromosomes containing *c-fes* and *c-abl* might correspond to those with known translocations or other rearrangements associated with certain human cancers.

To determine the chromosomal location of the human cellular homologue of *v-fes*, we initially screened restriction endonuclease-digested cellular DNAs from a series of mouse–human somatic cell hybrids by molecular hybridization. These hybrids, which contained a full complement of mouse chromosomes, but retained limited numbers of different combinations of human chromosomes, were analysed for the presence of the human *v-fes*-cross-reactive *EcoRI* restriction fragment. Identification of the human chromosomes present in the hybrids was based on isoenzyme, antigenic and chromosome analysis (Table 1). For identification of *c-fes* sequences, we used a ³²P-labelled probe corresponding to a 0.5-kilobase (kb) *PstI* restriction fragment localized within acquired sequences of the Snyder–Theilen FeSV genome, designated *v-fes* P_{0.5}. This probe has been described previously^{2,21} and is shown diagrammatically in Fig. 1. A single 12.0-kb *EcoRI* restriction fragment of human DNA could be distinguished from the major 8.5-kb *v-fes*-cross-hybridizing *EcoRI* fragment in mouse cellular DNA which migrated at a position close to that of a much less cross-reactive restriction fragment of mouse DNA at ~11.5 kb (Fig. 1). In preliminary experiments using this probe, the human *c-fes* was identified only in those hybrid clones containing human chromosome 15 (Table 1).

To obtain a molecular probe having increased specificity for the human *c-fes* gene, two 0.5-kb *KpnI* human restriction fragments (*c-fes* K_{0.5}) were isolated from a previously described cosmid clone containing the complete 12.0-kb *c-fes* *EcoRI* restriction fragment. Use of these restriction fragments as molecular probes considerably improved detection of the human *c-fes* 12.0-kb *EcoRI* restriction fragment in DNA both from human cells and from mouse–human somatic cell hybrid clones (Fig. 1, lanes *c–h*). As shown for representative hybrids in Fig. 1 and summarized in Table 1, the 12.0-kb *EcoRI* *c-fes*-homologous restriction fragment identified in this way was again observed only in DNA isolated from hybrid clones containing human chromosome 15.

Particularly compelling was the positive reaction with Hor19D2 hybrid DNA with the 0.5-kb *KpnI* human *c-fes* probe (Fig. 1, lane *e*). This hybrid contains human chromosomes 11, 15 and X as its only human contribution. Segregants of the Hor19D2 containing only the X chromosome (Hor19X) (lane *h*) or only chromosome 11 (Hor1 9D2RI; lane *f*) failed to react with the probe. The segregant Hor1-I which retained human chromosome 15 and a human–mouse translocation which includes part of the long arm of chromosome 11, also reacted strongly with the *c-fes* K_{0.5} (lane *g*) and *v-fes* P_{0.5} (Table 1) probes. The results of further restriction endonuclease analysis of DNA from segregant Hor1-I are shown in the right panel of the upper gel in Fig. 1. Two major *BamHI* restriction fragments of the human *c-fes* gene which hybridize with the *c-fes* K_{0.5} probe (lane *b*) are both identified in segregant Hor1-I. Similarly, two *SstI* fragments of 3.6 and 7.5 kb were identified in human DNA (lane *b*) and in the Hor1-I segregant (lane *c*), but not in DNA of mouse (lane *a*) origin.

An analogous approach was pursued to establish the chromosomal localization of the human cellular homologue of *v-abl*. In this case *EcoRI*-restricted DNAs from a similar series of mouse–human somatic cell hybrids were analysed. The molecular probes used included a *BamHI* (B_{3.5}) restriction fragment containing all but the 3'-terminal portion of the Abelson MuLV genome and a 1.7-kb *PstI* (P_{1.7}) restriction fragment localized within the Abelson MuLV-acquired cellular (*v-abl*) sequences (Fig. 1). A total of seven *EcoRI* restriction fragments of human DNA, ranging in size from 2.5 to 12.0 kb, and several of mouse DNA, were identified, each of which hybridized to the P_{3.5} probe. The five lower molecular weight human fragments were completely separated from mouse *c-abl* *EcoRI* restriction fragments, thus providing a means of identifying hybrid clones containing the human *c-abl* gene. The results of a typical analysis are shown in the lower left panel of Fig. 1 (lanes *a–c*). In other studies the complete human *v-abl* cellular homologue has been cloned from a cosmid library and at least

six of the seven *EcoRI* restriction fragments map to a single position. As an independent means of identifying hybrids containing the human *c-abl* gene, a 0.6-kb *BamHI* probe was prepared from the second lowest molecular weight (2.9 kb) *EcoRI* human restriction fragment and used as a probe. Representative data obtained with this probe are shown in the right panel of Fig. 1.

As summarized in Table 1, complete concordance was observed between the presence of the five *v-abl* cross-reactive *EcoRI* restriction fragments in the 2.5–5.0-kb size range and human chromosome 9, while each of the other chromosomes could be excluded by one or more examples of discordance. For example, human *c-abl*-specific restriction fragments were present in hybrid F4Sc13 Cl12 which contained only four human chromosomes: 1, 9, 14 and X. Of these, all but chromosome 9 were present in at least one hybrid which lacked the human *c-abl* gene, thus by elimination identifying chromosome 9 as the location of human *c-abl*. Finally, a series of four previously described hamster–human somatic cell hybrids were analysed for human *c-abl* sequences. As summarized in Table 1 and shown in the lower portion of Fig. 1 (lanes h–k), only one clone, C10b, contained human *c-abl*-specific sequences. Interestingly, this hybrid was positive for each of three isoenzyme markers for human chromosome 9, cytoplasmic soluble *cis*-aconitase (ACONs), AK3 and AK1. A subclone of C10b, designated C10b2BU, lacked detectable human *c-abl* sequences and by isoenzyme analysis was positive for only ACONs and AK3, both of which are localized on the short arm of chromosome 9. The third marker, AK1, which was not detected in this hybrid, is in contrast, localized to the long arm of chromosome 9. The remaining two hybrids were nonreactive in enzyme assays for all three markers for chromosome 9 and

lacked detectable sequences hybridizing to the human *c-abl* B_{0.6} probe.

These findings establish the independent chromosomal localization of human cellular homologues of two viral transforming genes, both of which encode tyrosine-specific protein kinases. The possibility that these functionally similar genes are all organized in a cluster such as that observed for the γ -globin, immunoglobulin or histocompatibility gene families is thus ruled out. Although the exact number of human genes encoding proteins with tyrosine-specific protein kinase activity remains to be determined, transforming proteins encoded by each of at least four independent avian type C viral oncogenes with such activity have been identified¹. One of these, *v-fps*, is structurally related to and maps at the same human cellular locus as the *c-fes* gene analysed here^{4,5}. Further study will be required to determine whether human cellular homologues of any of the three other avian viral oncogenes map to the same positions as either *c-fes* or *c-abl*.

In other studies, the mouse *c-abl* gene has been assigned to mouse chromosome 2 (S. P. Goff, P. D'Eustachio, F. Ruddle and D. Baltimore, personal communication). It is interesting that the human homologues of several linked markers assigned to mouse chromosome 2 are found on the long arm of human chromosome 9, indicating that these are, at least in part, homologous linkage groups²². The present results obtained from analysis of the hamster–human hybrids suggest that *c-abl* could be on the long arm of human chromosome 9.

Our findings are of interest in that both *c-fes* and *c-abl* map on chromosomes which are frequently involved in specific translocations associated with human neoplasms^{23,24}. For example, a high percentage of human chronic myelogenous leukaemias (CML) involve translocations of the distal portion of the long

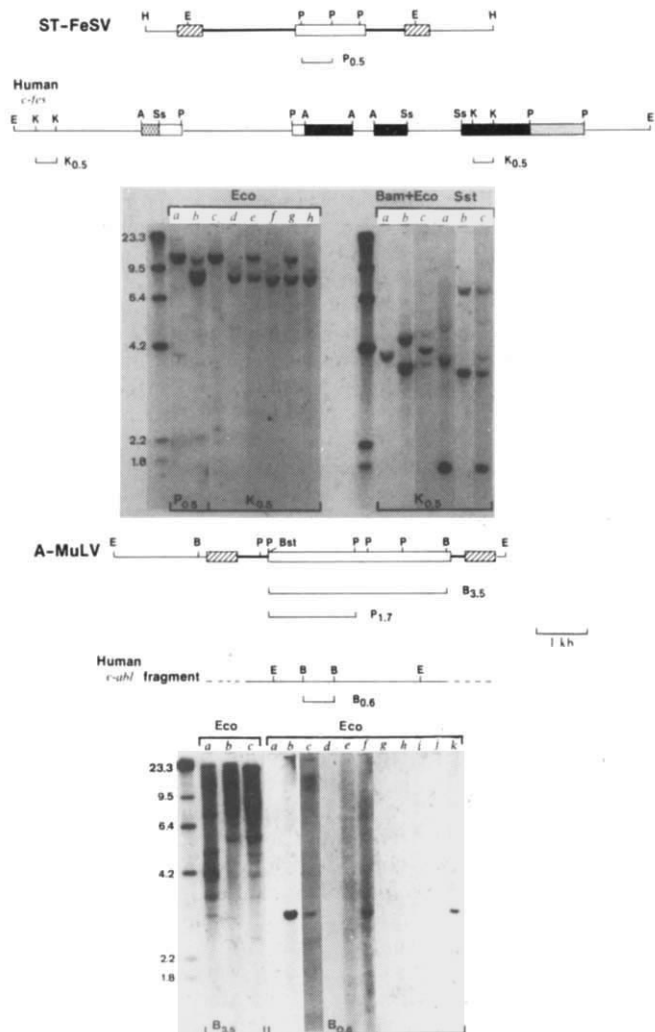


Fig. 1 Identification of human chromosomes containing *v-fes*- and *v-abl*-homologous sequences. Molecular clones of the ST-FeSV and human *c-fes* sequences, shown diagrammatically at the top of the figure, are based on previously published data³. The open box (□) within the viral genome represents the position of the acquired cellular sequences, while the feline leukaemia virus cross-reactive sequences are shown as solid lines (—). The positions of the long terminal repeats (▨), cellular flanking sequences (□) and the 0.5-kb *PstI* restriction fragment used as a probe (P_{0.5}) are also shown. The organization of the human *c-fes* has been published previously². For reference, the positions of previously identified restriction fragments of the human *c-fes* gene with homology to ST-FeSV, GA-FeSV and the Fujinami strain of avian sarcoma virus, as well as two 0.5-kb *KpnI* restriction fragments which were co-purified and used as a probe (K_{0.5}), are shown. Restriction patterns of cellular sequences hybridizing to the *v-fes* and *c-fes* probes are shown in the uppermost gel. Cell lines from which DNAs were prepared are described in detail in Table 1 legend and include in the left panel: b, d, NIH/3T3(–) mouse; a, c, A673 human(+)⁴⁰ and mouse–human hybrids; e, Hor19D2(+); f, Hor19D2R1(–); g, Hor1I(+); h, Hor19x(–); in right panel: a, NIH/3T3(–) mouse; b, A673 human(+); c, mouse–human hybrid Hor1I(+). (+ Indicates a positive assignment; – indicates absence of the particular chromosome.) Molecular probes and restriction enzymes are as indicated. Molecular clones of the Abelson MuLV (A-MuLV) genome and a region of human *v-abl*-homologous sequences are shown diagrammatically in the centre of the figure. Restriction fragments used as hybridization probes, including 1.7-kb *PstI* (*v-abl* P_{1.7}) and 3.5-kb *BamHI* (*v-abl* B_{3.5}) restriction fragments as well as a 0.6 kb *BamHI* (*c-abl* B_{0.6}) restriction fragment mapping within the 2.9-kb *EcoRI* human *c-abl* restriction fragment, are indicated. Restriction patterns of cellular DNA sequences hybridizing to the *v-abl* B_{3.5} and *c-abl* B_{0.6} probes are shown in the lower portion of the figure. Cell lines shown in the left panel include: a, A673 human(+); b, NIH/3T3 mouse(–); c, mouse–human hybrid F4sc13C112(+). Those in the right panel include: a, NIH/3T3 mouse(–); b, A673 human(+); mouse–human hybrids: c, F4sc13C112(+); d, FIR5R3(–); e, F4sc13C19(–); f, F4sc13C112(+); g, hamster; hamster–human hybrids; h, D1S20(–); i, C10b2BU(–); j, C4a(–); k, C10b(+). The derivations of these cell lines and their complements of human chromosomes are described in detail in Table 1 legend. Following hybridization with the P_{0.5}, B_{3.5} and P_{1.7} probes, filters were washed with 2.5×SSC at 65°C; using K_{0.5} and B_{0.6} probes, washes were performed using 0.3×SSC at 65°C according to the method of Bernards and Flavell⁴¹. Restriction enzymes are abbreviated as follows: E, *EcoRI*; H, *HindIII*; P, *PstI*; K, *KpnI*; A, *AvaI*; Ss, *SstI*. *HindIII*-digested λ DNA is included as a molecular weight standard.

Table 1 Hybridization of DNA from human-mouse and human-hamster somatic cell hybrids with *c-abl*- and *c-fes*-specific probes

Hybrid	Ref.	Result	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	X		
a <i>c-fes</i>																											
MOG-2	27	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
Dur4.4	28	+	-	-	-	-	-	-	-	-	-	-	+	+	+	+	+	-	-	-	-	+	+	+	+	+	
CTP41 P1	29	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
Hor19D2	-	+	-	-	-	-	-	-	-	-	-	-	+	-	-	-	+	-	-	-	-	-	-	-	-	+	
Hor19X	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	
Hor19D2R 1	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	+	
Hor1 I	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	
MOG 13/22	30	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-	+	+	+	
b <i>c-abl</i>																											
MOG-2	27	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
MOG-2 E5	31	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	
MOG-2 G1	31	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
SIR-7 A2	31	-	+	+	-	-	+	-	+	-	-	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	
SIR-7 D1	31	-	+	+	-	-	+	-	+	-	-	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	
SIR-7 G1	31	-	+	+	-	-	+	-	+	-	-	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	
Hor19D2	32	-	-	-	-	-	-	-	-	-	-	-	+	+	-	+	+	-	-	-	-	-	-	-	-	+	
F4Sc13C112	-	+	+	-	-	-	-	-	-	-	-	+	-	-	-	-	+	-	-	-	-	-	-	-	-	+	
F4Sc13C19	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	+	
MOG-13/22	30	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	
FIR 5R3	33	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	+	-	-	-	-	-	+	
Dur4.3	28	-	-	-	-	-	+	NT	-	-	-	-	-	-	+	+	+	-	+	-	+	-	+	+	+	+	
D1S20	34	-	NT	NT	NT	NT	NT	NT	NT	NT	-	-	NT	+	-	NT	+	NT	-	NT	-	NT	NT	NT	NT	NT	
C10b	35	+	NT	NT	+	NT	NT	NT	NT	NT	-	+	+	-	-	NT	+	NT	-	NT	-	NT	NT	+	+	+	
C10b2BU	-	-	NT	NT	+	NT	NT	NT	NT	NT	-	(+)	-	-	-	NT	+	NT	-	NT	-	NT	NT	NT	tr	-	
C4a	35	-	NT	NT	-	NT	NT	NT	NT	NT	+	-	-	-	-	NT	-	NT	-	NT	-	NT	NT	NT	-	-	

The origin and details of the initial characterization of the somatic cell hybrids listed in column 1 are described in the references indicated in the second column. Some of these hybrids have been subcloned after the initial characterization. (C10b2BU is bromodeoxyuridine-selected subclone isolated from C10b. F4Sc13 C19 and F4Sc13 C12 were subclones, the original cells were a gift from Dr H. Koprowski. These hybrids were isolated from a fusion of human fibroblasts and RAG mouse cells. Hor19D2 is a clone of Hor19 (ref. 32) with an identical karyotype. Hor19D2R1, Hor1I and Hor19X are subclones of Hor19D2.) Four of the hybrids described, including D1S20, C10b2BU, C4a and C10b, represent human-hamster somatic cell hybrids; all remaining are human-mouse hybrids. The chromosomal content was deduced from a combination of karyotypic, antigenic and enzymatic analyses. Karyotypic analysis was done by a combination of G11 staining and quinacrine banding^{36,37}. Techniques and markers for antigenic analyses are reviewed in ref. 38. Assays for human enzymes were performed using standard methods³⁹. Analysis of hybrids for *c-fes* and *c-abl* sequences was performed as described in Fig. 1 legend using *v-fes* B_{0.6} and *c-fes* K_{0.5} probes in *a* and *v-abl* B_{3.5} and *c-abl* B_{0.6} probes in *b*. Discordance between the presence of any one chromosome and hybridization with probes for either locus is shown by open boxes. Karyotypic analysis of F4Sc13C112 indicates that only about 4% of the cells contain chromosome 1. NT, not tested.

arm of chromosome 22 to chromosome 9. The resulting abnormal form of chromosome 22 has been called the Philadelphia chromosome. The long arm of human chromosome 9 which is specifically involved in this rearrangement is the region of human chromosome 9 that appears to correspond to mouse chromosome 2. Similarly, a translocation involving the long arm of chromosome 15 has been observed in cells of patients with acute promyelocytic leukaemia (APL)^{23,24}. Abelson MuLV was initially derived from a spontaneously occurring lymphosarcoma of the mouse and induces lymphoid tumours *in vivo* inoculation²⁵. Although *c-fes* was derived from a fibrosarcoma of the cat²⁵, the possibility that its disease spectrum in natural conditions and in the human population may be very different from that as a recombinant virus must be considered.

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It will thus be of interest to examine levels of expression of *c-fes* and *c-abl* in tumour cells with translocations involving chromosomes 15 and 9, respectively, and to establish by *in situ* annealing techniques whether these genes are, in fact, near the specific translocation points found in chromosome 9 for CML and in chromosome 15 for APL.

Since this work was completed, Dalla-Favera *et al.*²⁶ have assigned *c-fes* to chromosome 15.

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Sequence of an HLA-DR α -chain cDNA clone and intron-exon organization of the corresponding gene

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The HLA-DR and related antigens are the major cell-surface glycoproteins identified so far as involved in regulation of the immune response in humans¹. Here we present and discuss the sequence of cloned cDNA copies of the mRNA for the HLA-DR α -chain². The sequence is 1,195 nucleotides long, with one open reading frame encoding a 254 amino acid primary translation product. After cleavage of the signal sequence this yields a mature polypeptide of 229 residues. Four introns have been located within the corresponding genomic sequence revealing a correlation between the protein domain structure and the genomic exon organization. Analysis of the HLA-DR α -chain amino acid sequence shows a clear homology with HLA-ABC, β_2 -microglobulin and immunoglobulin domains.

The structure of the cDNA insert of clone pDRH2 is shown in Fig. 1, and the corresponding nucleotide sequence is shown in Fig. 2. Starting from position 27, with an ATG initiation codon, there is a continuous stretch of 762 nucleotides in an open reading frame, encoding 254 amino acids. The known N-terminal sequence begins at position 102 with the isoleucine codon ATC^{2,3}, suggesting that there is a signal sequence^{4,5} of 25 predominantly non-polar amino acids, and giving a mature product of 229 amino acids. The remaining 5'-untranslated region in pDRH2 is incomplete.

Beginning with amino acid residue 192 there is a strongly hydrophobic sequence of 23 amino acids, which has all the expected features of a transmembrane region. The resulting 191 amino acid extracellular sequence corresponds reasonably well with the size estimated from papain water-soluble fragments of the HLA-DR antigens⁶. This leaves a cytoplasmic portion of 15 amino acids, from position 214 to the C-terminus at 229, which contains several hydrophilic residues, as is characteristic of the cytoplasmic segments of other transmembrane glycoproteins, such as HLA-ABC, H-2K and IgM⁷⁻⁹. The sequence shown in Fig. 2 has two potential glycosylation sites conforming to the canonical asparagine-X-(threonine or serine) sequence, at amino acid residues 78 and 118 as expected from previous biochemical analysis. There are two cysteine residues at positions 107 and 163 in the extracellular part of the HLA-DR α -chain, which are presumably available for disulphide bonding and correspond quite closely in their relative positions to the cysteine pairs found, for example, in the membrane proximal domain of HLA-ABC and other related glyco-

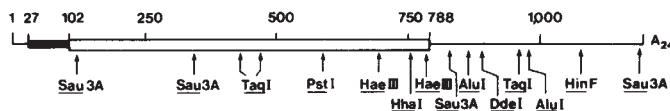


Fig. 1 Schematic diagram showing the structure of the cDNA coding for the HLA-DR α -chain cloned in pDRH2. Nucleotide residues are numbered in the 5' to 3' direction in the same orientation as the mRNA, beginning at the first nucleotide following the 17 guanine residues that were used to insert the cDNA into the *Pst* site of pAT153². The signal sequence (solid bar) begins at the ATG initiation codon in position 27–29, and the codon for the N-terminal amino acid, isoleucine, is at position 102–104. The coding region (open bar) extends to nucleotide 788, followed by the 3' untranslated region of 402 nucleotides before the poly(A) extension, which is 24 residues long in pDRH2. The terminal G and C extensions are not represented. The *Pst*I and *Sau*3AI cDNA-specific fragments were separated by electrophoresis in 9% polyacrylamide gels, cut out of the gels and eluted²⁹. The isolated fragments were used in all further experiments, whether or not subjected to cleavage by other enzymes before end labelling. The only restriction enzyme sites shown are those that were labelled at the 5' or 3' ends. Unique end labelled fragments were then obtained by strand separation²⁹ or by cleavage with another restriction enzyme followed by electrophoresis in polyacrylamide gels and dilution as already described. Nucleotide sequence of an extensive series of overlapping fragments was determined by the chemical degradation procedure of Maxam and Gilbert²⁹ and confirmed on *Pst*I–*Taq*I fragments inserted into M13mp7 phage by the chain termination technique³⁰.

proteins. This clearly suggests a two domain overall structure for the extracellular portion of the HLA-DR α -chain in which the N-terminal domain has no cysteine pairs, while the membrane proximal region is perhaps an immunoglobulin-like domain with a characteristic disulphide bond.

The amino acid sequence given in Fig. 2 is in general agreement with the partial sequence given by Korman *et al.*¹⁰, with the exception of valine instead of leucine at position 217. This difference may be genuine, since a change from G to T in the first nucleotide of the valine codon GTG, to produce leucine codon TTG, would be difficult to account for by cloning or sequencing artefacts.

Following the C-terminal stop codon TAA at nucleotide positions 789–791 there is a 404 nucleotide 3'-untranslated sequence, before the poly(A) sequence. The 3'-untranslated region shown in Fig. 2 appears to be a complete non-coding region as there is a consensus polyadenylation signal AATAAA starting 28 nucleotides upstream from the poly(A) at position 1,168. There is an additional polyadenylation signal in the same orientation starting a further 102 nucleotides upstream (position 1,066), which may occasionally be used (unpublished observations), as has been found in other situations.

To obtain cloned HLA-DR α -chain related genomic sequences we screened a cosmid library, made from human placental DNA (F.G., unpublished), with a labelled pDRH2 DNA probe. Out of several positive cosmids, one (10ii) contained a strongly hybridizing 3.4-kilobase (kb) *Eco*RI fragment which included most of the pDRH2 sequences, but did not contain the 5'-untranslated region or the signal sequence for the HLA-DR α -chain (data not shown). Further studies, to be reported in detail elsewhere, have now shown that in addition to the gene corresponding to the pDRH2 cDNA insert on cosmid 10ii, there are other related (but significantly different) sequences on chromosome 6 (unpublished data). Figure 3 shows that the first identifiable intron whose 3' junction sequence conforms to the consensus for a splice site, occurs within the codon for amino acid 3 (before nucleotide 109). There may, of course, be introns further upstream within the signal sequence and 5'-untranslated region, but these can only be located by analysing the sequences upstream of the 5' terminus of the 3.4-kb fragment, which are not present in cosmid 10ii. The second and third introns fall within the codons for amino acids 85 and 179 respectively. These introns define two exons corresponding

Table 1 Per cent homologies between various HLA-associated and immunoglobulin domain sequences

	DR α	DR β	HLA-ABC	β_2 m	C λ	C κ
DR α	—	35	31	29	22	19
DR β		—	30	33	25	21
HLA-ABC			—	21	21	18
β_2 m				—	17	16
C λ					—	35
C κ						—

The sources of the sequence data were DR β , ref. 17; HLA-ABC, the B7 sequence (S. Weissman, personal communication); β_2 microglobulin, ref. 31, C κ and C λ , ref. 21.

to the proposed N-terminal domain and the immunoglobulin-like membrane proximal domain respectively.

The last intron to be identified occurs 10 nucleotides downstream from the first stop codon, defining an exon that includes mainly the transmembrane and cytoplasmic portion of the HLA-DR α -chain. It is interesting that this last splice moves

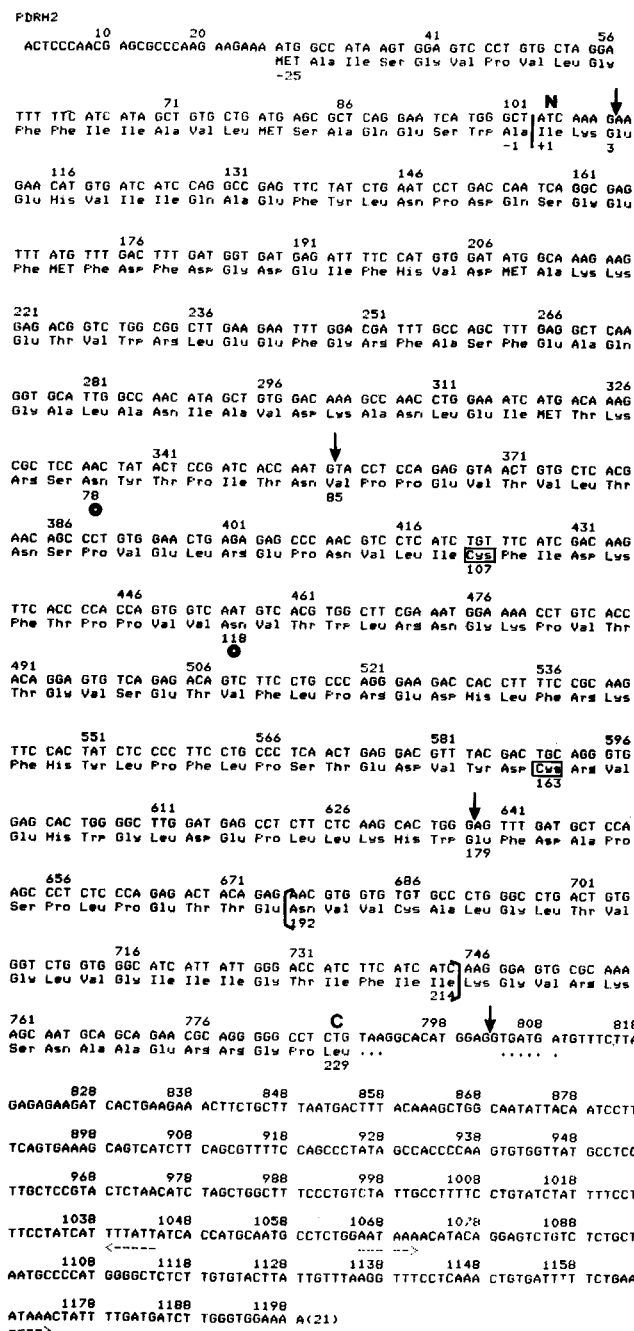


Fig. 2 The complete nucleotide sequence of cDNA insert of pDRH2 and the predicted amino acid sequence of the HLA-DR α -chain. The nucleotide sequence was determined as explained in Fig. 1 legend and follows the same numbering scheme. The derived amino acid sequence is shown below the nucleotide sequence, and numbered below from -25 to -1 for the signal sequence, and from 1-229 for the mature HLA-DR α -chain. The two cysteine residues that are presumed to form the interchain S-S bond are indicated by boxes; N and C indicate the amino, and carboxy termini respectively; ● indicate the glycosylation sites; arrows above the nucleotide sequence designate the known intron-exon boundaries (see text and Fig. 4); and the vertical lines after amino acids 191 and 214 delineate the transmembrane (TM) hydrophobic region. The in-phase termination codons are indicated by dots, and the poly(A) addition sites by arrows indicating direction.

two more nonsense codons at nucleotide positions 804-809 into phase. The remainder of the 3'-untranslated region is completely contained in the last exon (data not shown), but no sequence in the 3' flanking region in the genomic DNA has yet been determined.

A number of protein sequence studies have revealed homologies between various immunoglobulin domains, β_2 -microglobulin and HLA-ABC and H-2K D domains¹³⁻¹⁶, as had been predicted^{11,12}. More recently the membrane proximal external domain of HLA-DR β -chains has been added to this list¹⁷⁻¹⁹ and from the sequence data given in Fig. 2 the membrane proximal external domain, α_2 , of HLA-DR α -chains can also be included in this set of homologies.

The α_2 domain of the HLA-DR α -chain, defined by the splice points within the codons for amino acids 85 and 179 (see Fig. 2), was compared with the corresponding membrane proximal external domains of HLA-DR β and HLA-ABC chains as well as with β_2 microglobulin and the constant regions of human κ and λ immunoglobulin light chains. In addition to the pair of cysteines, proline residues at positions 87 and 114, phenylalanine at 112, tyrosine at 161, valine at 165, and his-

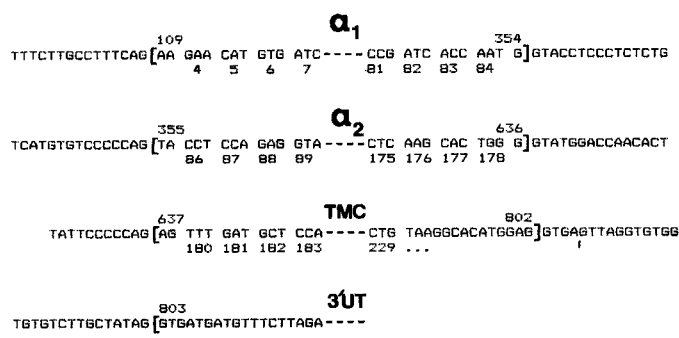


Fig. 3 Nucleotide sequences at the identified intron-exon junctions. The 3.4-kb *EcoRI* fragment from cosmid 10ii was first subcloned in pAT153 using standard techniques, and then *Sau3AI* and *EcoRI-PstI* fragments of the insert were further subcloned into the single-stranded DNA phage M13 to locate the intron-exon junctions within the genomic DNA by sequence determination. Templates with pDRH2 cDNA related sequences were identified by hybridization of dot-blots with the labelled cDNA insert. Several of these phages were then sequenced by the chain termination technique³⁰. α_1 and α_2 refer respectively to the exons encoding the N-terminal and membrane-proximal external domains. TMC refers to the transmembrane and cytoplasmic exon. Numbers above the sequence indicate nucleotide position and below indicate amino acid positions corresponding to those in Fig. 2. The three in-phase codons are underlined with dots.

tidine at 167 are common to all the domains compared in Table 1, and in fact to nearly all immunoglobulin constant region domains^{15,16,20-22}. These conserved residues may have some important structural role in maintaining the characteristic immunoglobulin fold²¹.

A number of homologies are found among the HLA associated domains themselves, which are more closely related to each other than they are to immunoglobulins. For example, all of the HLA related sequences have tryptophan at position 178. Moreover, in the HLA-DR α_2 domain, β_2 microglobulin and the HLA-ABC α_3 domain, this codon occurs one base before the intron between this exon and the exon encoding the transmembrane region. The valine at position 85 in the HLA-DR α -chain contains the splice site at the start of the α_2 exon and is also present in the HLA-DR β -chain sequence. These splice sites may lie in the same positions in the β -chain, a possibility further emphasised by the fact that the aspartate in the homologous position in the HLA-ABC α_3 domain²³ and the arginine in β_2 microglobulin²⁴ also contain the upstream splice sites for the respective domains. Other conserved sites of interest for the HLA associated set of domains are the proline at position 155 and the phenylalanine at position 145.

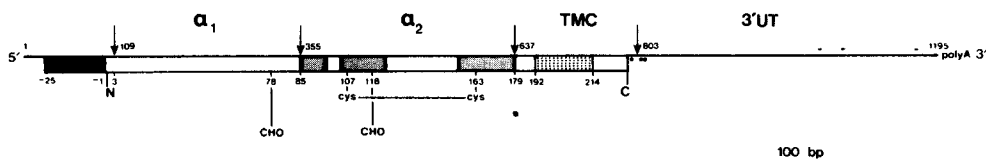


Fig. 4 Schematic summary of the organization of the HLA-DR α -chain sequence. The summary is based on the data of Figs 1–3 and follows their numbering and conventions. α_1 , α_2 , and TMC refer to the N-terminal, membrane-proximal, transmembrane-cytoplasmic exons or domains respectively, and 3'-UT refers to the 3' untranslated region exon. The rectangle identifies translated sequence. The solid region is the signal sequence, the shaded regions are those with greatest homology to other domains, and the stippled region is the hydrophobic transmembrane segment.

A schematic summary of the organization of the HLA-DR α -chain sequence based on the data of Figs 1, 2 and 3 is given in Fig. 4. The most obvious features revealed by the sequence analysis are the organization of the HLA-DR α -chain into exons that correspond surprisingly well to those defined for HLA-ABC chains, β_2 microglobulin and, of course, immunoglobulins, and the demonstration of a remarkable correspondence between exon organization and the presumptive protein domains. Another striking feature of the HLA-DR α -chain sequence data is the homology of the external domain next to the membrane with other comparable domains of HLA products, with β_2 -microglobulin and, to a lesser extent, with immunoglobulin constant domains. The fact that this homology applies to comparisons between specific domains, such as those close to the membrane, underlines the relative independence of the evolution of different domains, or exons. Though the homologies between the HLA products (ABC, DR α and DR β -chains) are weak compared with homologies between HLA-ABC and H-2KDL, or between the latter and the H-2 region coded *TL* and *Q* gene products, they are sufficient to support the suggestion that the whole, or at least a major part, of the HLA region may ultimately have a common origin by a series of duplication events²⁵. The implied separation of the different classes of HLA products by duplication must, however, have occurred up to 500 million years ago, and certainly well before the evolutionary divergence of the mammals²⁶.

The suggestion that there might be homology between HLA products and immunoglobulins was made on the basis of a functional analogy, namely the possibility that HLA products might have recognition functions analogous to those of the variable regions of immunoglobulins^{11,12}. Subsequently, however, it became clear that while HLA products do play a part in cellular interactions and recognition, at least in the immune system, they do not have an analogue of the immunoglobulin variable region. Thus, it is not surprising that homologies between HLA and immunoglobulins revealed by sequence analysis involve particularly the constant region domains, as might be expected if HLA products are involved in interactions similar to those that occur between immunoglobulins and complement²⁵. Fc receptors, monocyte receptors and secretory components²⁷ as well as between complement and cellular component receptors. Another relevant analogy might be that between the C_H3 domain of IgG and the appropriate receptor on specific antibody dependent killer cells²⁸.

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Electron microscopy of *t*-allele synaptonemal complexes discloses no inversions

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The *t* complex of mice has been an enigma for over half a century¹. The recessive mutations (*t*ⁿ) on chromosome 17 of the mouse have been characterized by four properties: interaction with the dominant mutation *T* (*Brachyury*) to cause taillessness, transmission ratio distortion in males, a series of homozygous lethal mutations, and cross-over suppression^{2–4}. The last property has led to many studies of meiosis in *t*-allele-bearing males. Forejt found fewer chiasmata, the cytological correlate of crossing-over, in the region of *t* haplotypes^{5,6}. Lyon *et al.*⁷ confirmed these results and suggested that chiasma suppression determined by *t* alleles is probably desynaptic but that electron microscopy would probably be needed to settle this point. We have now extended these observations by using a centromeric translocation and analysed synaptonemal complexes by electron microscopy to study *t*-haplotype pairing. We have found normal synaptonemal complexes without any evidence of inversions but detected early disjunction of the chromosome 17 homologues, supporting the idea of desynaptic chiasma suppression.

The cross-over suppression of *t* haplotypes masks the true genetic nature of the region. It has previously been convenient to consider the *t* alleles as point mutations mapping near *T*.

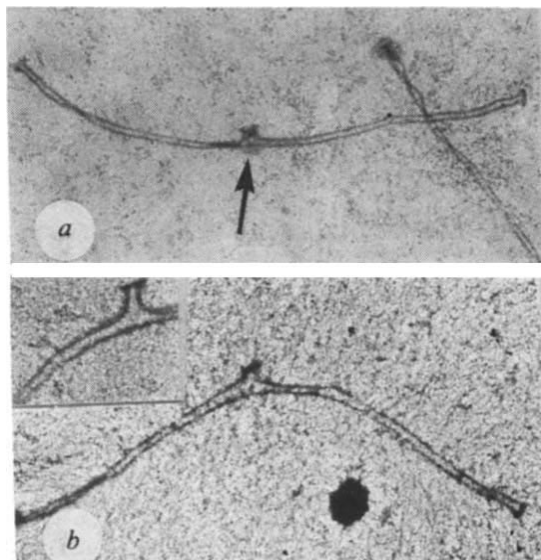


Fig. 1 Whole-mount electron microscopic preparations of mouse pachytene spermatocytes. Testicular cells were dissociated and suspended in phosphate-buffered saline as described previously¹⁷. Nuclear contents were dispersed in a detergent (NP40) solution and transferred to a plastic trough containing electron microscope copper grids and a solution of 0.1 M sucrose with 10% formaldehyde. The sample was centrifuged at 3,000 r.p.m. for 5 min at 4 °C. Grids were rinsed in 0.5% Kodak Photo-flo, air-dried and stained with 1% phosphotungstic acid in 95% ethanol. A full description of this technique has been reported elsewhere¹⁷. The criteria for identification of meiotic prophase stages in whole-mount electron microscopic preparations have been described in ref. 18. *a*, Whole-mount electron micrograph of an early pachytene mouse spermatocyte displaying a synaptonemal complex between robertsonian translocation 7 and chromosomes 16 and 17 (*Rb7/t^{w5}*). The arrow indicates the paracentromeric chromatin region. $\times 4,600$. *b*, Pairing of chromosomes 16 and 17 mediated by a synaptonemal complex in a mouse spermatocyte carrying *Rb7/T*. $\times 6,200$. Inset: detail of the telomeric pairing of chromosomes 16 and 17 in the presence of *Rb7/T*. $\times 11,000$.

Lyon's thorough analysis of this chromosomal region using rare cross-overs established the non-allelism of several *t*-complex properties^{8,9}, and the apparent size of the *t* complex was estimated by deletion mapping to be at least 6 centimorgans^{10,11}. More recently, Artzt, Bennett and co-workers have used increased cross-over frequencies, which occur in heterozygotes containing two different haplotypes, to suggest that the *t* complex may be 15 centimorgans or more long¹² and involves a reverse order of *H-2* and *tufted*¹³. A chromosomal inversion would readily explain the *t*-complex cross-over suppression. There have been previous reports of chromosomal aberrations (interpreted as deficiencies)¹⁴ in this region of chromosome 17 but inversions were not found¹⁵.

We have used a robertsonian translocation of chromosomes 16 and 17 (*Rb7*, ref. 16) as a marker for studies of the synaptonemal complex in the *t* region by silver staining of light microscopic preparations and by electron microscopy. *Rb7*, previously designated *T7Bnr*, was obtained from Dr Charles J. Epstein in homozygous condition on the C57BL/10J background. *F₁* mice from the cross of the balance lethal *T/t^{w5}* (originally obtained from Dr Hugh McDevitt) and *T/t¹²* (originally obtained from Dr Salome Gluecksohn-Waelsch) lines were known to be *Rb7/t^{w5}* (or *Rb7/t¹²*) if they had normal tails while *T/Rb7* males had short tails. Spermatocytes were prepared by detergent solubilization of cell membranes and dispersion of nuclear contents which were then collected on microscope slides or electron microscopy grids^{17,18}. The whole-mount electron microscopic preparations were examined in a JEM 100 B transmission electron microscope operated at an accelerating voltage of 60 kV. Chromosome 16 and 17 monovalents formed normal synaptonemal complexes with the

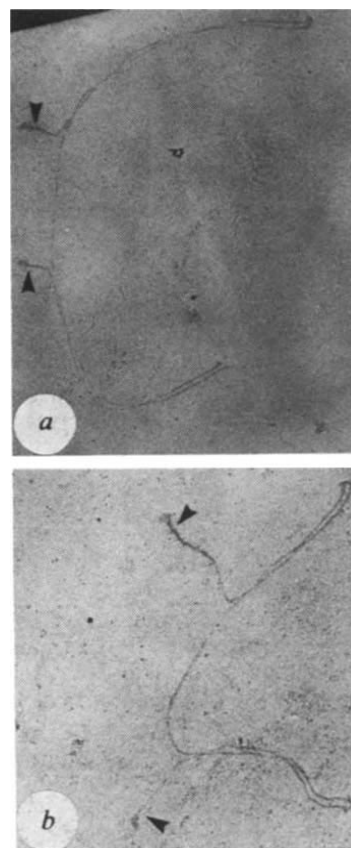


Fig. 2 Whole-mount electron microscopic preparations of mouse spermatocytes (middle pachytene) from *Rb7/t^{w5}* males. See Fig. 1 legend for preparation details. Early disjunction of chromosomes 16 and 17 is observed at the centromeric regions, including the chromosomal segment corresponding to the *t* complex. Arrowheads indicate early disjunction. *a*, $\times 4,000$; *b*, $\times 5,000$.

Rb7 bivalent; the centromeric region seemed to pair and project on one side of the complex (Fig. 1*a*). Very similar configurations have been described for silver-stained trivalents in the male cotton rat where a balanced robertsonian polymorphism occurs¹⁹. These synaptonemal complexes in *t*-haplotype-bearing mice were identical to those observed in the sibling *T/Rb7* controls (Fig. 1*b*). In middle pachytene spermatocytes, early disjunction of the centromeric regions of chromosomes 16 and 17 were observed in the presence of *t* haplotypes (Fig. 2*a, b*). This early disjunction of both chromosomes 16 and 17 in the trivalent was seen equally with *t¹²* (Fig. 3). They were also noted in silver-stained preparations of the pachytene spermatocytes (Fig. 4).

Thus, normal synaptonemal complexes occur between *t*-haplotype chromosomes 17 and their normal homologues. However, early disjunction of the paracentromeric regions of

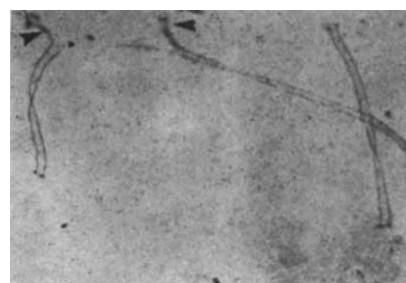


Fig. 3 Middle pachytene spermatocyte, prepared as described in Fig. 1 legend. Early disjunction of *Rb7/t¹²* at the paracentromeric region is indicated by arrowheads. $\times 4,800$.

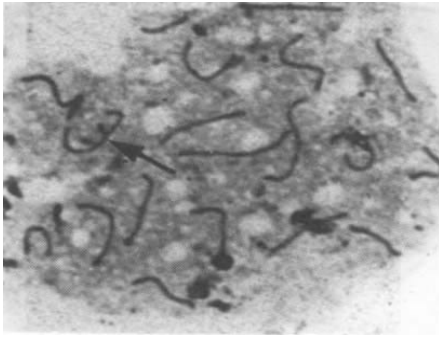


Fig. 4 Silver-stained light microscopic preparation of a pachytene mouse spermatocyte (*Rb7/t^{w5}*) displaying synaptonemal complexes of different lengths. Arrow indicates early disjunction of chromosomes 16 and 17 at the paracentromeric region. The preparation was according to ref. 17. $\times 1,430$.

chromosomes 16 and 17 occurs in the presence of the *t* haplotype. This phenomenon may be related to the *t* haplotype and not to *Rb7*, because early disjunction does not occur in the *T/Rb7* sibling controls. Our electron microscopic studies also support the assumption of Lyon *et al.*⁷ that chiasma suppression in *t* haplotypes results from normal synapsis without chiasma formation (desynapsis).

Artzt, Shin and Bennett have found an anomalous position of *H-2* with respect to *tufted* and *t*-haplotype lethality in *t^{w5}*, one of the *t* haplotypes used in this study¹³. The position could be most readily explained by an inversion of chromosome 17. Such inversions have not previously been found. Our electron microscopic and silver-staining studies of synaptonemal complexes have shown no evidence of inversions in either *t^{w5}* or *t¹²*. As the *t* complex may extend for about 15 centimorgans, inversion loops would be expected if they were of this size—inversion loops of synaptonemal complexes were found in 100% of inversion carriers at early stages of meiosis²⁰. Alternatively, the apparent change of position of *H-2* in these *t* alleles could represent expression of previously unexpressed type 1 histocompatibility antigen loci—recent evidence suggests much greater dispersion of type 1 loci, for at least several centimorgans, than had previously been expected²¹. Despite the central role of crossing-over suppression for an understanding of the *t* complex, our electron microscopic analysis of synaptonemal complexes has not shown any clear-cut abnormalities. Thus, the final resolution of the enigma of the *t* complex must await the availability of DNA clones of the region.

Although our studies do not reveal the mechanism of *t*-allele-mediated cross-over suppression, further chromosomal studies are warranted as cross-over suppression and transmission ratio distortion in males, not embryonic lethality, are the truly unique properties of the *t* complex. The frequency of developmental lethals is probably no greater than expected in 15 centimorgans of DNA—the cross-over suppression maintains a block of DNA which can accumulate lethals and the unique population-selection properties of the *t* complex outbalance the loss due to homozygous lethality²². Recent discoveries of novel *t* alleles in novel complementation groups²³ suggest that previous developmental descriptions, based on a small number of complementation groups, have given a mistaken notion of molecular relatedness among these developmental lethals. Thus, all *t* lethals, and not just *t^{w5}* (ref. 24), may be 'parasitic'.

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Cooperative motion and hydrogen exchange stability in protein β -sheets

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Protein molecules are dynamical structures due to the continual exchange of thermal energy between them and the solvent environment^{1,2}. This dynamic behaviour is manifest in hydrogen exchange experiments, which reflect transient solvent accessibility of groups usually buried in the protein interior^{3,4}. However, studies of hydrogen exchange kinetics in pancreatic trypsin inhibitor (PTI) reveal a small subset of amide protons which exchange very slowly^{5,6}. Four of these groups form successive interchain hydrogen bonds in the central region of an antiparallel β -sheet⁷ (Fig. 1). Here I suggest that the unusual exchange stability of these β -sheet protons reflects the structure's intrinsic flexibility. This property allows transient energy fluctuations to be accommodated as cooperative motions which do not locally strain the interchain hydrogen bonds.

Observed hydrogen exchange rates for proteins typically span several orders of magnitude³, which may reflect both intrinsic and environmental differences among the individual exchanging groups^{8,9}, as well as their static or dynamic accessibility to solvent molecules⁴. Nevertheless, examination of either the geometries^{7,10} or solvent accessibilities¹¹ of the PTI sheet hydrogen bonds suggests little apparent reason why protons in the centre of the sheet should exchange more slowly than other similarly inaccessible groups (Fig. 1). Indeed, the data suggest that whatever the amplitude of dynamical motions in the fluctuating molecule^{1,2}, the central-sheet hydrogen bonds rarely break, as required to make them both accessible to, and reactive with, an exchanging water molecule^{3,4,9}.

Features of the antiparallel β -sheet which give rise to its flexibility are illustrated in Fig. 2, which shows a double-stranded sheet with linear N—H—O—C interchain hydrogen-bonds¹² (conformation 1 on the ϕ , ψ plot of Fig. 3). The structure can be viewed as a set of interconnected large and small hydrogen-bonded rings, each of which possesses a local dyad symmetry axis owing to the antiparallel sense of the polypeptide chains. This 'classical' structure can be continuously compressed in accordion-like manner so that it retains its symmetry properties¹³. This involves equivalent alteration in all the backbone ϕ , ψ angles and isoenergetic bending of the hydrogen-bonds^{12,13} at the carbonyl oxygens (that is, conformations 1–4 in Fig. 3). Similarly, each flat sheet conformation can twist to

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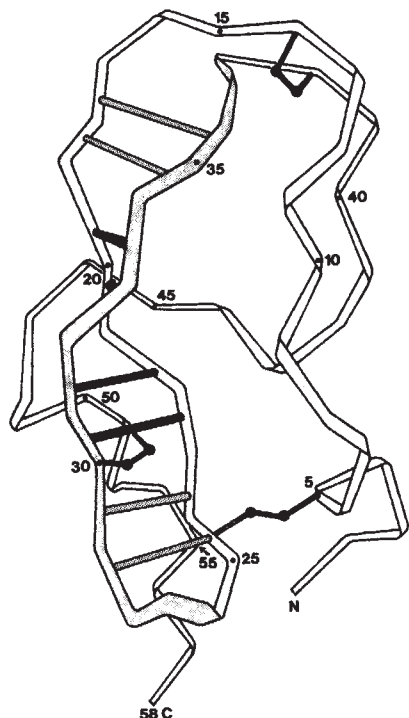


Fig. 1 A ribbon drawing of pancreatic trypsin inhibitor emphasizing the double-helical, antiparallel β -sheet (shaded) incorporating residues 17–36. Of the groups forming interchain hydrogen bonds in the sheet (illustrated as rungs interconnecting the polypeptide chain), four in the centre of the sheet (solid rungs) incorporate peptide amide groups which are unusually resistant to exchange with solvent protons, whereas those at the sheet ends exchange much more rapidly. Nevertheless, due to packing interactions of amino acid side chains, all the sheet hydrogen-bonding groups are similarly inaccessible to solvent in the crystal structure (for example, see Fig. 9 of ref. 13).

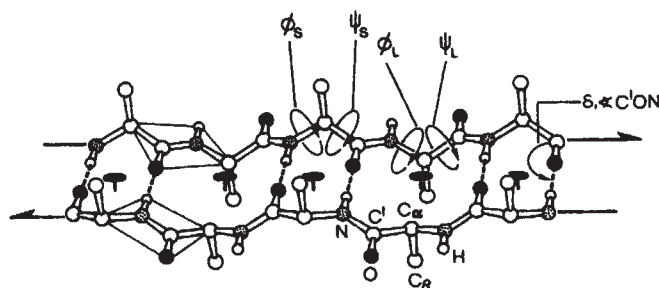


Fig. 2 A section of antiparallel β -sheet. The flat structure consists of 2-fold helical polypeptide chains linked by interchain hydrogen bonds. Due to the antiparallel N to C sense of the chains, the extended structure is organized as an interconnected array of 'small' and 'large' hydrogen-bonded rings, each of which has a 2-fold rotational symmetry axis normal to the mean sheet plane. The principal low-energy degrees of freedom of the backbone structure are torsional rotations about covalent backbone single bonds (ϕ_s , ψ_s , ϕ_L , ψ_L), and hydrogen-bond bending at the peptide carbonyl oxygen (δ).

form right-handed double-helical structures, corresponding to minimum energy conformations for extended polypeptide chains of L-amino acids^{14,15}. In this case the chains coil so that the ϕ s and ψ s of residues in small and large hydrogen-bonded rings behave in a different, but correlated fashion. This double-helical coiling again preserves the ring symmetry in the structure, and equivalently, the interchain hydrogen bonds¹³. Due to the independence of the 'accordion' and coiling motions, the structure can undergo continuous interconversion between any of the structures shown in Fig. 3 via correlated motions associated with hydrogen-bond preservation. This reflects an extra-

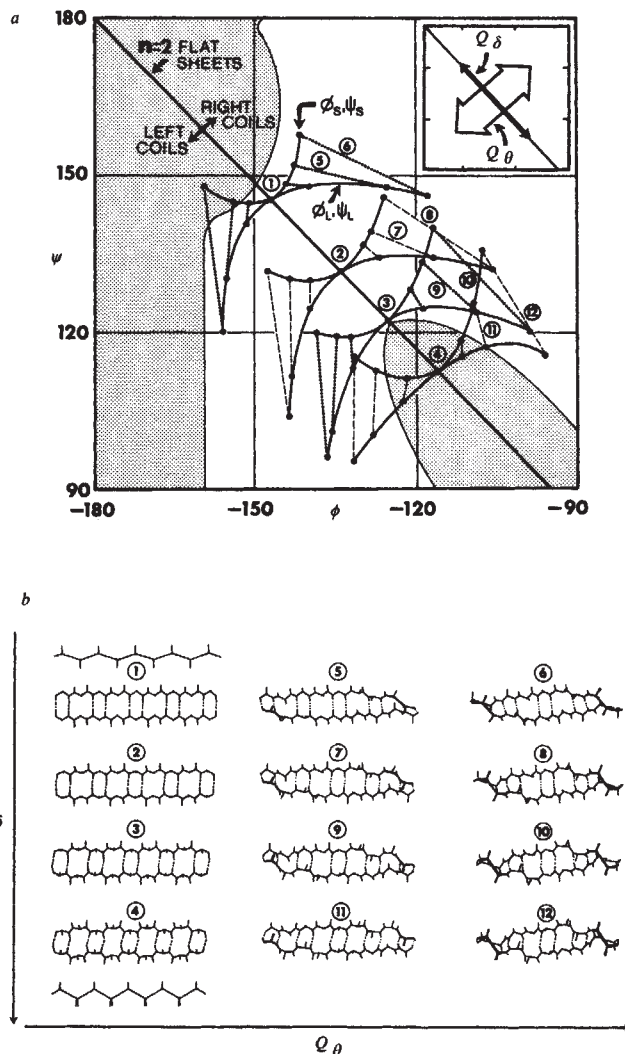


Fig. 3 a Shows the extended chain region of the ϕ , ψ plot. Various flat sheets are composed of 2-fold helical polypeptide chains having identical ϕ , ψ values for all residues. A continuum of flat-sheet conformations lie along the $n = 2$ line of the plot and differ in their hydrogen-bond bend angles (δ in Fig. 2), as shown by corresponding numbered structures in b. Any flat structure can cooperatively twist to form double-helical structures. This involves different, but correlated alterations in the ϕ , ψ angles of residues situated in small and large rings of the sheet. The twisted structures shown in b therefore consist of residues whose ϕ , ψ values alternate so that small ring residues all have conformational values ϕ_s , ψ_s , and large ring values ϕ_L , ψ_L , corresponding, respectively, to pairs of ϕ , ψ values connected by dashed and dotted lines in a. Shaded regions in a approximately delineate sterically disallowed sheet conformations. The accordion compression (Q_δ) and coiling (Q_θ) motions can be viewed as cooperative oscillation modes of the structure as a whole. Analysis of the potential energy surface¹⁵ governing the coupled oscillations of the twisted sheet is consistent with a structure of unusual conformational flexibility.

ordinary extent of conformational flexibility for an extended protein secondary structure. For example, comparable concerted ϕ , ψ excursions severely deform or sever the hydrogen-bonds in an α -helix¹⁶.

As described elsewhere¹³, the crystallographically observed β -sheet in PTI is well approximated as a symmetrical and highly twisted structure of the type shown in Fig. 3. The observed structure therefore approximates a time-average superposition of the cooperative coiling (Q_θ) and accordion (Q_δ) motions, undergoing small excursions about their potential minima. Consequently, it might be anticipated that the same cooperative

interactions apparent in the crystallographic structure are retained when the molecule undergoes infrequent, larger fluctuations in energy. This suggests that fluctuations which might otherwise result in local structural disruptions promoting hydrogen exchange^{3,4,8}, can be accommodated in concerted β -sheet motions which incur little dynamic stress on the interchain hydrogen bonds.

As outlined above (see Fig. 3), the flexibility of the antiparallel β -sheet is a fundamental reflection of its symmetry properties, which allow equivalent preservation of the interchain hydrogen bonds for large cooperative displacements in its lowest-energy, torsional degrees of freedom (that is, ϕ , ψ rotations). However, the PTI sheet differs from the idealized model as the strands are interconnected by a β -turn at one end, and sharply folded over at the other (Fig. 1). In the present context, these interactions at the sheet ends can be viewed as imposing constraints on symmetrical oscillations characterizing the remainder of the structure. In other words, excursions in the symmetrical modes would be expected to localize dynamic stresses, and hence frequently break hydrogen bonds, at the sheet ends, where the structure deviates most from the idealized symmetry. This behaviour is consistent with the observed exchange properties in PTI, where the hydrogen bonds at the sheet ends (Fig. 1) exchange much faster than those in the centre^{5,6}.

Although dynamical simulations^{17,18} of PTI cover time intervals many orders of magnitude shorter than that generally associated with hydrogen exchange processes³, they show properties consistent with the behaviour described here. Specifically, these simulations give evidence for both correlated oscillations about the β -sheet backbone bonds¹⁷ and preservation of the interchain hydrogen bonds in the sheet centre¹⁸. Figure 3 illustrates that these properties are retained even during infrequent fluctuations, resulting in significant displacements from the structure's minimum free energy state.

These observations suggest that the unusual exchange stability observed in the PTI β -sheet, and also seen in more extended antiparallel sheets in trypsin¹⁹ and ribonuclease²⁰, reflects cooperative oscillations in these structures as a whole. Such cooperative motions may be relevant to protein folding, stabilization and function²¹. For example, the coupled motions described suggest highly cooperative pathways for β -sheet folding. Similarly, oscillations in the folded structure, corresponding to thermal excitation of low-frequency vibrational modes²¹, may contribute a favourable vibrational entropy component to the molecule's stabilization free energy²². Finally, these cooperative motions are ordered both in space and time, features implicit in most present proposals for enzyme allostery and mechanism.

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Redesigning enzyme structure by site-directed mutagenesis: tyrosyl tRNA synthetase and ATP binding

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We describe here a general method for systematically replacing amino acids in an enzyme. This allows analysis of their molecular roles in substrate binding or catalysis and could eventually lead to the engineering of new enzymatic activities. The gene encoding the enzyme is first cloned into a vector from which the enzyme is expressed and is then mutated *in vitro* to change a particular nucleotide and hence the amino acid sequence of the enzyme. We have cloned the gene for the tyrosyl tRNA synthetase of *Bacillus stearothermophilus* into a vector derived from the single-stranded bacteriophage M13 to facilitate mutagenesis with mismatched synthetic oligodeoxynucleotide primers. From the recombinant M13 clone we have obtained high levels of the enzyme (~50% of soluble protein) expressed in the *Escherichia coli* host and have converted cysteine (Cys 35) at the enzyme's active site to serine. This leads to a reduction in enzymatic activity that is largely attributable to a lower K_m for ATP.

The tyrosyl tRNA synthetase (TyrTS) catalyses the aminoacylation of tRNA^{Tyr} with tyrosine by a two-step mechanism in which tyrosine is first activated by ATP to form tyrosyl adenylate and then transferred to tRNA^{Tyr} (refs 1, 2). The nucleotide sequence of the gene has been determined from a clone in the plasmid vector pBR322³ and the enzyme is expressed in *E. coli* from its own promoter to about 10% of total soluble protein⁴. X-ray crystallographic analysis of the tyrosyl enzyme is complete to ~3 Å resolution⁵ and the binding sites of tyrosine and ATP have been identified^{6,7}. The target for mutagenesis was Cys 35⁸, which is also present in the structures of the tyrosyl and methionyl tRNA synthetases of *E. coli*⁹. In the crystallographic model of the tyrosyl enzyme, this residue makes a contact with the 3' hydroxyl of the sugar moiety of tyrosyl adenylate, possibly via a hydrogen bond (D. Blow, personal communication). We decided to replace this cysteine by serine, a close structural analogue, expecting that a conservative change in a binding contact might alter, but not destroy, enzyme activity.

Initially the gene encoding the tyrosyl tRNA synthetase and its promoter were re-cloned from pBR322⁴ into the single-stranded bacteriophage M13 to facilitate mutagenesis¹⁰ (Fig. 1). The principle of oligonucleotide-directed mutagenesis relies on the extension of a primer by DNA polymerase (large fragment on a single-stranded circular template¹¹⁻²⁴). The primer, 5' CAAACCCGCTGTAGAG 3', was complementary to the template except for a single internal mismatch (*) which directs the mutation Cys (TGC) to Ser (AGC). The mismatch is sited in the middle of the primer to protect it from exonuclease action and the precise sequence and length of the primer was chosen to minimize spurious matches elsewhere in the gene or in M13. The newly synthesized strand was sealed with ligase and *E. coli* cells transfected with the cloned circular double-stranded molecule.

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Single-stranded phage was isolated from individual plaques, spotted on to nitrocellulose and the mutants identified by hybridization with 5' 32 P-labelled mutagenic primer. At room temperature the primer anneals to both mutant and wild-type DNA but by washing at a higher temperature the primer can be selectively dissociated from wild-type DNA^{15,16}. A short wash at $T_D + 2^\circ\text{C}$ (T_D is the dissociation temperature calculated by the rule $T_D = 2^\circ$ per dA · dT and 4° per dG · dC nucleotide pair¹⁷) was sufficient to remove selectively most mismatched primer from wild-type DNA. Of the 36 plaques screened, at least 16 contained mutant phage (Fig. 2). Phage from two of these samples were plaque-purified to ensure the complete segregation of wild-type and mutant molecules, and re-hybridized with primer to identify pure mutant plaques. The required mutation was confirmed by dideoxy sequencing¹⁸ of the pure mutant template with a restriction fragment primer adjacent to the site of mutagenesis (not shown). The directed nature of the mutagenesis should exclude mutations at other sites, therefore we did not sequence the rest of the mutant gene. Neverthe-

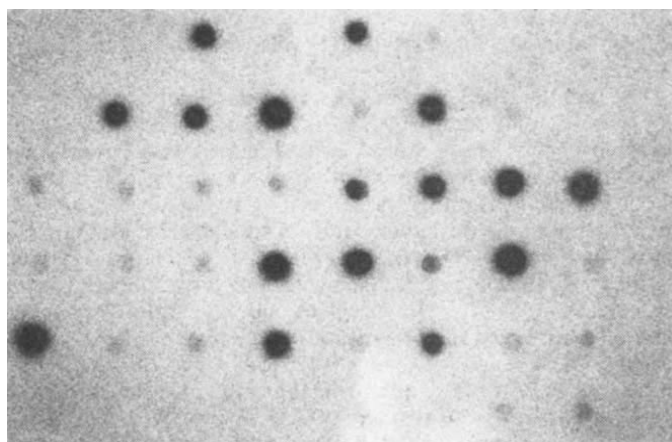


Fig. 2 Screening mutant phage by dot-blot hybridization: 36 plaques were grown in 1-ml cultures and phage prepared as described elsewhere³⁴. Phage were dissolved in 50 μl water and for hybridization³⁵, 1 μl of each was spotted on to nitrocellulose (Schleicher & Schull) and the filter baked at 80°C for 1 h. After pre-hybridization with $6\times\text{SSC}$, $10\times\text{Denhardt's}$ solution³⁶, 0.2% SDS at 67°C for 1 h, the filter was rinsed with 50 ml of $6\times\text{SSC}$. The filter was hybridized at room temperature for 1 h by leaving it in an open Petri dish containing 2 ml of $6\times\text{SSC}$, $10\times\text{Denhardt's}$ solution and 10^6 c.p.m. (Cerenkov) probe. The probe was prepared by treating 20 pmol of mutagenic primer with kinase as described in Fig. 1 legend, except that the following were used: 2 units T4 kinase, no cold ATP and $10\ \mu\text{Ci}$ [$\gamma\text{-}^{32}\text{P}$]ATP ($>2,000\ \text{Ci}\ \text{mmol}^{-1}$; NEN), followed by desalting on a 5-ml column of Sephadex G25F. The filter was rinsed in 50 ml $6\times\text{SSC}$ for 1 min and placed between two sheets of Saranwrap (Dow). Autoradiography for 1 h revealed that each clone had hybridized to the probe. The filter was then washed in pre-warmed $6\times\text{SSC}$ for 4 min at 52°C (this temperature corresponds to $T_D + 2^\circ\text{C}$). (Previously, we washed the filters at several intermediate temperatures to selectively remove the primer¹⁰, and although the $T_D + 2^\circ\text{C}$ rule has worked for several primers we do not know whether it is general.) Autoradiography overnight revealed that 16 of the 36 phage preparations contained mutant; two of these preparations were plaque-purified and six plaques from each were grown up as described above and re-screened to identify pure mutant phage.

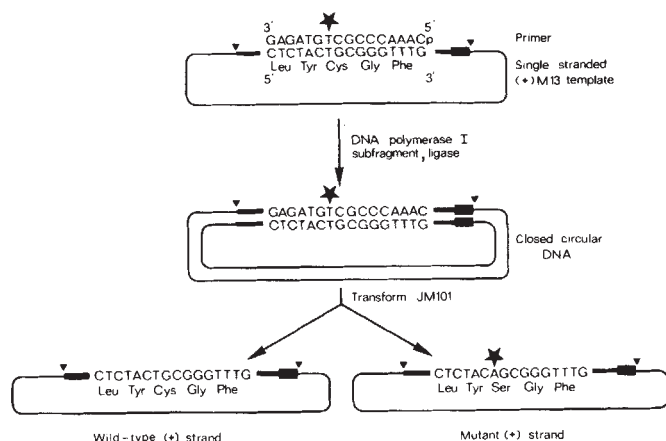


Fig. 1 Scheme for primer-directed *in vitro* mutagenesis of tyrosyl tRNA synthetase. The tyrosyl tRNA synthetase gene was cloned as a 2.5-kilobase(kb) insert into the *Bam*HI site of pBR322 after partial *Sau*3A digestion of *B. stearothermophilus* chromosomal DNA⁴. A 2.5-kb *Sa*II fragment was excized from the TyrTS-pBR322 recombinant plasmid and re-cloned into M13mp93 (J. Messing, unpublished) vector in the orientation indicated. The fragment contains the TyrTS gene (1,257 base pairs, bp) flanked by ~1,000 bp at the 5' end and 296 bp at the 3' end: the 3' portion includes 184 bp from pBR322 corresponding to the section between the *Bam*HI and *Sa*II sites of the plasmid³⁰. ▽, The limits of the insert; the thickened line indicates the *B. stearothermophilus* DNA; ■, the portion of pBR322. Mutagenesis was essentially as described elsewhere¹⁰; 100 pmol of 5' CAAACCGCTGTAGAG 3' primer (Applied Biosystems) was phosphorylated with 6 units of T4 kinase (BRL) in 30 μl 0.1 M Tris-HCl pH 8.0, 10 mM MgCl₂, 100 μM ATP, 5 mM dithiothreitol (DTT) at 37°C for 50 min and then heated at 70°C for 10 min. Phosphorylated primer (12 pmol) was annealed with 0.5 pmol of TyrTS-M13 template in 10 μl 20 mM Tris-HCl pH 7.5, 10 mM MgCl₂, 50 mM NaCl, 1 mM DTT at 55°C for 5 min, then cooled to room temperature. The volume was adjusted to 20 μl 20 mM Tris-HCl pH 7.5, 10 mM MgCl₂, 25 mM NaCl, 0.5 mM DTT, 0.5 mM dTTP, 0.5 mM dCTP, 0.5 mM dGTP, 0.5 mM ATP, and the primer extended for 5 min with 2 units of DNA polymerase I large fragment (BRL) at limiting dATP (2.5 μM , $10\ \mu\text{Ci}$ [$\alpha\text{-}^{32}\text{P}$]dATP, NEN) in the presence of 3 units of T4 DNA ligase (BRL). dATP was then increased to 0.5 mM, the reaction incubated at 15°C for 18 h and then stopped by adding 100 μl 10 mM Tris-HCl pH 8.0, 10 mM EDTA. After phenol extraction, an equal volume of 13% polyethylene glycol, 1.6 M NaCl was added to the aqueous phase and the sample was kept on ice for 10 min³¹. DNA was separated from unincorporated [$\alpha\text{-}^{32}\text{P}$]dATP label by a 10-min spin in an Eppendorf microfuge and the DNA pellet was dissolved in 200 μl 0.2 M NaOH. Most of the label (80%) had been incorporated into DNA. After 5 min at room temperature the sample was applied to a 5 ml alkaline sucrose gradient (5–20% sucrose, 1 M NaCl, 0.2 M NaOH, 2 mM EDTA) and centrifuged in a Beckman SW50.1 rotor at 37,000 r.p.m., 4°C for 2 h. Aliquots (6 drops) were collected from the bottom of the tube; the closed circular (cc) DNA (19% incorporated [$\alpha\text{-}^{32}\text{P}$]dATP) runs ahead of single-stranded linear and circular molecules. The ccDNA was neutralized with 1 M Tris-citrate, dialysed against 10 mM Tris-HCl pH 8.0, 0.1 mM EDTA and used to transform the *E. coli* strain JM101³² as described elsewhere³³; 1% of the ccDNA yielded ~500 plaques.

less we showed by kinetic analysis (see below) that extracts from the two independently cloned mutant plaques had the same specific enzymatic activities.

E. coli infected with wild-type or mutant phage was then checked for expression of the enzyme. A sonicate of cells revealed that the tyrosyl tRNA synthetase now constituted $>50\%$ of the soluble protein in infected cells (Fig. 3). Such high levels of expression either suggest that the synthetase promoter is strong or reflect the high copy number of M13 replicative form (RF) in the cell. The relative copy number of the vectors might account for the increased expression on re-cloning the gene from pBR322 (20 plasmid copies per cell¹⁹) into M13 (200 RF copies per cell²⁰). Whatever the causes of the high expression in M13, it is most useful and may have wider applications.

Aminoacylation assays on the cell extracts revealed that both mutant and wild-type extracts contained active enzyme, but that the extract from the mutant had a lower activity. The retention of significant activity in the mutant extract indicates that the enzyme does not strictly require a sulphhydryl group at this position. Furthermore, as Cys 35 is the only conserved cysteine in the sequences of the tyrosyl tRNA synthetases of *B. stearothermophilus*³ and *E. coli*²¹ it seems that thioacyl intermediates²² are probably not involved in the mechanism of these enzymes. For a more detailed kinetic analysis we measured the amount of enzyme [E_0] in each crude extract by active site titration involving the formation of tyrosyl adenylate²³. The specific activity ($V_0/[E_0]$) of the pyrophosphate exchange and aminoacylation reactions (Table 1) was thus calculated and the results showed that the mutant enzyme was intrinsically less active than the wild type. Further work indicated that on mutation, the value of K_m for ATP in aminoacylation increased from

0.9 to 4.1 mM while $V_{\max}$ was reduced (1.4 to 0.9 s⁻¹). The K_m for tyrosine in aminoacylation was, however, unchanged (15 μ M).

The fact that the lesion has no effect on tyrosine binding but does affect the binding of ATP is certainly consistent with the location of Cys 35 at the ATP-binding site in the crystallographic model. Why the substitution of serine for cysteine should result in reduced ATP binding is not clear from this experiment alone. Although serine is smaller than cysteine^{24,25} and may therefore not make an effective van der Waals' contact with the 3' hydroxyl of the sugar, it can form much stronger hydrogen bonds^{26,27}. However, as has been discussed elsewhere²⁸, such capability need not confer improved binding as the S—H—O and O—H—O hydrogen bonds have slightly different geometries^{24,25} and the OH group of Ser 35 could bind water molecules more strongly than does the SH of Cys 35. In fact the serine hydroxyl probably contributes no net binding energy, as the kinetics of a Gly 35 mutant are essentially identical to those of the Ser 35 mutant (unpublished work). Thus, the reduced energy of ATP binding on converting Cys 35 to serine is probably due to the loss of the van der Waals' contact. We therefore envisage that the molecular role of Cys 35 is to make optimal contact with the sugar moiety of the ATP.

Further mutagenesis experiments are planned to delineate the role of individual residues in binding ATP, tyrosine and tRNA and in the catalytic mechanism. The use of M13 vectors for both mutagenesis and expression of the mutant enzyme, and the use of active site titration to measure accurately the amount of enzyme in a crude sonicate of *E. coli*, will facilitate this approach.

We thank Dr D. G. Barker for the TyrTS-pBR322 clone, Dr J. Messing for the vector M13mp93 and Professor D. M.

Table 1 Activity of TyrTS in cell extracts

Extract of JM101 infected with	TyrTS (nmol of active sites)*	Pyrophosphate exchange rate (nmol s ⁻¹)	Aminoacylation rate (nmol s ⁻¹)
M13mp93	<0.02	0.5†	<0.05
M13mp93 + 2.8 nmol of purified TyrTS‡	2.8	7.4	2.7
TyrTS-M13	2.8	7.1§	3.2
TyrTS (Cys → Ser)-M13	2.9	2.1†	1.0

Total enzymatic activities in the extract of 20-ml cultures of infected cells. All kinetic experiments were performed at 25 °C in a standard buffer containing 144 mM Tris-HCl (pH 7.8), 10 mM MgCl₂, 0.1 mM phenylmethylsulphonyl fluoride and 10 mM 2-mercaptoethanol. The active site titration mixture²³ contained, in addition, 2 mM ATP, 10 μ M [¹⁴C]-Tyr (770 c.p.m. pmol⁻¹) and 10 U ml⁻¹ inorganic pyrophosphatase. The pyrophosphate exchange²⁹ mixture contained 2 mM ATP, 2 mM ³²P-PP_i and 50 μ M tyrosine. The aminoacylation mixture contained 20 μ M [¹⁴C]-Tyr (770 c.p.m. pmol⁻¹), 2 mM ATP, 20 μ M tRNA^{Tyr} (*B. stearothermophilus*, 200 pmol per A₂₆₀ tyrosine acceptance) and 10 U ml⁻¹ inorganic pyrophosphatase. One equivalent of Mg²⁺ was added together with ATP to maintain free Mg²⁺ at 10 mM.

* Determined by active site titration.

† In the absence of added tyrosine there was an exchange rate of 0.45 nmol s⁻¹.

‡ A sample of TyrTS purified from *B. stearothermophilus* was added to the extract of M13mp93-infected cells in the same proportion as produced in the cloned strains. Addition of extract had no effect on active site titration or aminoacylation rate and merely added the background of 0.4 nmol s⁻¹ to the pyrophosphate exchange rate.

§ In the absence of added tyrosine there was an exchange rate of 1.4 nmol s⁻¹.

Blow for making available unpublished data and the crystallographic model of TyrTS. M.S. is a career investigator of the MRC of Canada and the work was supported by the MRC of the UK and of Canada.

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Fig. 3 Expression of tyrosyl tRNA synthetase from M13 in *E. coli*; 1 ml phage stocks in 2×TY media were prepared from a single plaque as described elsewhere³⁴ and 50 μ l were used to inoculate 20 ml JM101 (D_{550} = 0.05) in 2×TY. Cells were grown for ~5 h until D_{550} = 1 and then 1 ml of lysate was prepared by freeze-thaw and sonication⁴; 50 μ l were analysed on a 10% SDS-polyacrylamide gel³⁷. Sonicates of: a, JM101 cells; b, JM101 infected with M13mp93; c, JM101 infected with wild-type TyrTS-M13; d, JM101 infected with mutant TyrTS-M13; e, TyrTS (*B. stearothermophilus*) marker purified as described in ref. 38.

BOOK REVIEWS

Memories may be beautiful, and yet . . .

Stuart Sutherland

IN THE opening chapter of *Memory Observed*, Professor Ulric Neisser excoriates experimental psychologists for studying memory only in the laboratory. He claims that with the exception of memories that last for less than a minute, such as sensory images, psychologists have discovered almost nothing about memory that is not known to eight-year-old American school-children. They must be bright youngsters since many of the findings are by no means obvious to adults. For example, learning a series of lists facilitates the learning of further lists but impairs memory for them; recall is facilitated if it takes place in the same environment as that in which learning occurred; and the main reason why recognition is easier than recall is that it requires less information.

Flourishing the cant phrase "ecological validity", Neisser argues that the most important aspects of memory can only be investigated by studying it in natural situations. It is perhaps as well for physics and chemistry that no advocates of ecological validity sprang up to hinder progress in these subjects. If one cannot understand what happens in a simplified laboratory situation, what hope is there of understanding "real life"?

After Neisser's tirade against conventional psychology, one turns eagerly to his selection of over 40 articles on memory in naturalistic situations, hoping that they will astound the eight-year-old child. In fact they are entertaining rather than surprising. They provide little that is of either practical or theoretical importance and nothing that is newsworthy. In his commentaries, Neisser himself does not attempt to extract general principles from the articles he reprints, but there appear to be four.

First, people often remember things that have no apparent relevance to their goals. One study showed that most Americans could remember what they were doing when they heard the news of John Kennedy's assassination. But man's capacity for remembering irrelevant material is surely notorious: any lecturer knows that his students are more likely to remember his jokes and asides or even the colour of his tie than the gist of his lecture.

Second, memory for things of interest may sometimes be very good. One study showed that the events surrounding the birth of a sibling are remarkably well retained, even by people who were only three years old when their brother or sister was born. Members of primitive and

Memory Observed: Remembering in Natural Contexts. By Ulric Neisser. Pp.433. Hbk ISBN 0-7167-1371-3; pbk ISBN 0-7167-1372-1. (W.H. Freeman: 1982.) Hbk \$24.95, £18.80; pbk \$12.50, £8.80.

illiterate tribes can remember numerous details of their tribal history or of totemic material and some specialize in this activity, but doubtless an equal display of virtuosity is exhibited by boys in many London schools in their knowledge of the heroes of association football.

Third, in contrast to the previous principle, memory is unreliable and people often think they remember better than they do. Experiments on the unreliability of eyewitness testimony have a long history. It is nice but hardly surprising to know that if a witness has seen a snap-shot of someone, it increases the chance of his picking out that person as "the criminal". A recent American study shows that few Americans can remember in detail what is on the face of a cent. A much earlier British study, not cited, showed that almost nobody could correctly recall the position of the letters on a British telephone dial. One of the more entertaining articles is by Neisser himself. He compares the testimony given by John Dean to the Watergate Committee of the United States Senate with the taped record of the meetings in the White House that Dean was describing. Dean correctly reported the more salient implications of what was said, but despite his asseverations that he remembered the details, he was incorrect both in his recall of his own words and of those of Nixon. It is not very clear what this tells us of the way memory works, though it is a striking example of how we may deceive ourselves in our memories even of important occasions.

Fourth, memories may be distorted to fit in with what one would have liked to have happened. Some of Dean's errors appear to have been wish-fulfillments: he was confident that Nixon had praised him for his part in the Watergate cover-up, but the tapes show he was wrong. Several experiments show that people systematically distort their memory of previously held opinions to make them conform to their current attitudes.

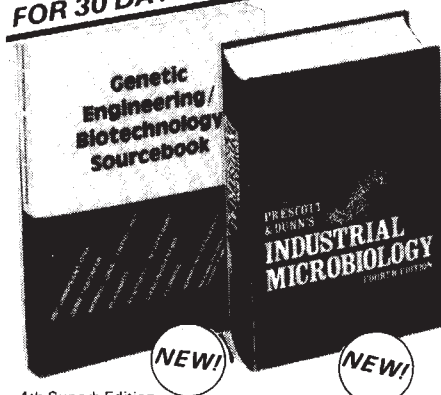
None of the above findings reveal anything not already known or predictable from laboratory experiments or indeed from common knowledge, though it would be interesting to know what determines when recall will be accurate and when it will be poor.

Neisser's final section is devoted to mnemonists or memorists as they are called in the United States. They remain a puzzle. Luria's "S" could remember at a single reading lists of over a hundred nonsense syllables and could recall them accurately sixteen years later. Anything he read produced synaesthetic images that were so intense they made it hard for him to follow the meaning. Just as remarkable is the recent case of "Elizabeth" who could form extremely detailed eidetic images. She could look at one half of a random dot stereogram with the left eye, memorize it, then memorize the other half with the right eye and on calling the two images back to memory could fuse them and see depth. Neisser also includes accounts of "Shass Pollaks" — Jewish scholars who have memorized the thousands of pages of the Talmud and can recall the words that appear on any line of any page. I liked the story of Toscanini who, on being told by his second bassoonist that the key to his instrument's lowest note was broken, thought for a moment and said "It's all right — that note does not occur in tonight's concert". Despite their fascination, it is hard to know what to make of the accounts of memorists. Their remarkable capacities appear to have little if any survival value and indeed in the case of "S", the mechanism on which his memory was based made daily living difficult for him.

It must be admitted that the study of long-term memory has advanced little since the war despite the vast amount of research done on it. With the exception of visual memory, there are no interesting theories of the structure of memory nor of how the elements of that structure are addressed to obtain recall. We do not know why memory should be so capricious. Nor do we know why intense pain or distress is so hard to recall. Perhaps a perfect memory would inhibit the imagination and the recall of previous pain would prevent us enduring again the situation that caused it. On the evidence of Neisser's book it is unlikely that our knowledge of memory will be advanced by naturalistic experiments. But neither will laboratory experiments advance our understanding until someone proposes a more comprehensive and interesting theory of memory than any that have yet been put forward. □

Stuart Sutherland is Director of the Centre for Research on Perception and Cognition at the University of Sussex.

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Vitamin C. The Mysterious Redox-System — A Trigger of Life? By Silvia Nobile and Joan Mary Woodhill. Pp.185. ISBN 0-85200-419-2. (MTP Press: 1982.) £11.95, \$29. *Vitamin C in Health and Disease.* By T. K. Basu and C. J. Schorah. Pp.152. UK ISBN 0-07099-0445-2; US ISBN 0-87055-406-9. (Croom Helm/Avi: 1982.) £11.95, \$19.50. *Vitamin C (Ascorbic Acid).* Edited by J. N. Counsell and D. H. Hornig. Pp.383. ISBN 0-85334-109-5. (Applied Science: 1982.) £32.

FASCINATING stuff vitamin C: it makes you ill if you don't eat it. And if you believe what you read in these books it cures almost every affliction known to man: not just scurvy, but colds, cancer and cardiovascular disease. It also apparently needs talking about over and over again, which is why there are three recent books to review instead of just one.

Three new books might seem three too many. After all vitamin C is already a bit of a nutritional cliché. The dietary sources are so well known even nutritionists don't need to look them up. The assay for it is so simple, it is part of almost all nutrition courses — even school-children are taught to measure vitamin C (and consequently discover just how little there is in their overcooked school lunches) and the deficiency disease has been controllable for nearly two centuries. Since, in fact, James Lind's famous experiments with the British Navy which provided a steady market for West Indian limes and an apparently indestructible nickname for the English.

The real justification for these books is the notion that old hat and practically unimportant as the antiscorbutic properties of vitamin C may be, it also has a different incarnation, one of importance to us all since it means this vitamin though chemically only a simple carbohydrate, is a physiological wonder, the key to many of the uncured ills of our age.

Superficially the books seem very different; Counsell and Hornig are the editors of a symposium, Basu and Schorah are authors of an undergraduate textbook, and Nobile and Woodhill . . . well, what can be said about a book whose subtitle is *The Mysterious Redox-System — A Trigger of Life?*

Anyone buying the book to find out about life's trigger will be disappointed. It is mysterious because it doesn't actually appear in the text, and vitamin C as an *in vivo* redox system is only dealt with briefly and obscurely. However, most of the book provides a competent introduction to this most over-discussed vitamin, although unfortunately the authors don't seem quite sure who they are writing for. I found that their literary style lacked consistency and was in places convoluted, elsewhere reminiscent of Enid Blyton.

Basu and Schorah are presumably competing for the same book market, though they do so by producing a more terse and more conventionally "scientific" monograph. What spoils it for me is that their enthusiasm and commitment to the importance of vitamin C in human health results in a somewhat atrophied critical faculty. For example, they reproduce on page 96 a graph, ascribed to Ginter, which, they argue, shows that in guinea pigs the rate of transformation of cholesterol to bile acids is related to hepatic vitamin C concentration. Actually it shows that in one group of vitamin C deficient guinea pigs with hepatic vitamin C concentrations in the range 0–4mg/100g, the rate of transformation ranged from 5 to 12mg/day/500g liver, while in another group of normal guinea pigs where the concentration ranged from 5 to 12, the rate of transformation ranged from 8 to 16. To my eye *within* neither group was any correlation evident, nor was the difference between the groups statistically significant. To Basu and Schorah it is the illustration of a proven fact, and the beginning of a section which ends by proposing that changes in vitamin C status are central in fluctuations in mortality from ischaemic heart disease.

My reservations about the last book in the trilogy, entitled simply *Vitamin C (Ascorbic Acid)*, are of a different kind. The book is solid science (apart from the re-appearance of Ginter's graph) and covers food science and analytical chemistry, as well as nutrition in a competent, plodding manner. But it has arisen out of a symposium organized and sponsored by Roche Products Limited and its editors work for the Roche empire, as do 5 of its 24 distinguished contributors who report on, *inter alia*, the prevalence of subclinical vitamin C deficiency and the safety of megavitamin C dosage.

This kind of commercial intertwining is inevitable in modern science but I wish that the book simply pointed out that Roche market vitamin C. And I wish that the book included a chapter on the boom that there has been in vitamin C self-supplementation as book after article after broadcast has advocated megavitamin therapy.

All the enthusiasm for vitamin C arises because human beings need it, and a major end product of all this scientific activity is persuading ordinary people that they need to take supplements of the stuff. A daily gram tablet of Redoxon in my local chemists costs over 7 pence and it sells well. I don't say that all discussions should start there, but I do think they should mention it. □

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Harnessing the power of genetics

P.A. Lawrence

Genetic Neurobiology. By Jeffrey C. Hall, Ralph J. Greenspan and William A. Harris. Pp.284. ISBN 0-262-08111-3. (MIT Press: 1982.) \$30, £20.95.

WHEN I was an undergraduate studying biology, genetics was not estimated as much use for anything — at least not by those who organized our timetable. Paramount were the sciences of chemistry, botany and zoology; these lectures were given in the morning when we were thought to be most receptive. Spent by the afternoon's sport a minority of us went at 5 p.m. to J.M. Thoday's excellent lectures on genetics, and tried to learn things that were deemed insufficiently important to feature in the exams.

This attitude to genetics has been all too common, and can still be easily detected. But genetics provides powerful tools to help us understand biology as, although the approach is almost bound to be indirect, it can lead us to the heart of intricate and difficult problems.

You don't need to read much of *Genetic Neurobiology* to discover that the authors are also fans of the genetic approach, and their book justifies their enthusiasm. It is not a multi-author work in the usual sense as the three authors have written all of it together; typical sections consist of a detailed description of a topic plus occasional bits of critical and thoughtful commentary. It is an instructive and useful mix because the reader can clearly see at what stage the field is, what needs to be understood and, sometimes, how the next step might be achieved.

The book is generally clear, entertainingly peppered with typos, and only occasionally becomes heavy going. My only minor complaint is that there are too many references to unpublished work; many unpublished discoveries remain unpublished for good reason.

A great deal of ground is covered: examples are movement in bacteria, sensory receptors in *Paramecium*, *Drosophila* and nematodes, vision, learning and courtship in fruit flies, cell lineage in *Caenorhabditis elegans*, neurogenesis in vertebrates, brain circuitry in Siamese cats and even psychosis in human beings. This might sound indigestible but adherence to the genetic approach focuses the material nicely.

The book contains many good examples of the power of genetic methods. Here is one: the biological clock has been around for a long time, we know that the behaviour of animals is subject to an internal timer but we do not know what makes the clock tick, or even if it does. In *Drosophila* a particular gene, *per*, is close to the heart of a "clock" which tells the flies that it is time to hatch out of their pupae (Konopka and Benzer, *Proc. Natn. Acad. Sci.* 68, 2112;

1971). Mutations in the *per* locus change the periodicity of this behaviour and have allowed the methods of mosaic fate mapping to locate the clock in the head. Lack of the *per*⁺ function appears to stop the clock and hatching becomes arrhythmic, one *per* mutation speeds up the clock, and another slows it down. These hatching times are circadian, but significantly the same mutations also affect a behaviour which cycles every minute or so — the courtship song of the male — and they affect the cycle length in the same way.

Mars viewed through Viking eyes

J. Veverka

The Surface of Mars. By Michael H. Carr. Pp.232. ISBN 0-300-02750-8. (Yale University Press: 1982.) \$45, £35.

THE 1976 Viking mission to Mars not only answered the age-old question, "Is there life on the Red Planet?", but provided a wealth of new information, much of it contained in the more than 50,000 images returned by the Viking Orbiters between 1976 and 1980. *The Surface of Mars*, written by Michael Carr, leader of the Viking Orbiter Imaging Team, is an excellent summary of what Viking saw. While the emphasis is on the surface and on its study through the Orbiter cameras, related topics and techniques are dealt with as needed to provide a comprehensive picture of the planet today and to outline its complex history.

Carr's book is a worthy successor to the *Geology of Mars* by Thomas A. Mutch and colleagues (Princeton University Press, 1976) which was based on Mariner 9's initial reconnaissance in 1971–1972. The discussion follows a logical progression: major surface features and processes are considered systematically, building up a detailed view of Mars. Throughout, there is an admirable tendency to concentrate on the big picture and shun side-issues. The treatment emphasizes questions rather than answers and conveys accurately the idea that Mars remains a puzzle in spite of Viking's thorough investigation.

Of all the surface features, perhaps the most enigmatic are the various dry valleys or "channels" originally discovered by Mariner 9. Usually attributed to erosion by liquid water, they have been used to argue that the martian climate was very different at some time in the past than it is today. (Currently the atmosphere is too thin and too cold to permit the presence of open bodies of liquid water.) Carr carefully discusses the types and probable ages of the channels and critically reviews various

With *Drosophila*, geneticists and molecular biologists have worked out how to take laborious but straightforward steps which allow one to go from mutants in a gene, to the cytological locus on the chromosome, to the gene itself and finally to the product. So the *per*⁺ product may be identified soon, and that could get us into the workings of the clock at last.

This book is a success and is well worth detailed study; and it is even entertaining — where else could you read about the mosaic wasp that spent its days stinging would-be mates and copulating with caterpillars? □

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suggestions for their origins. A serious problem, and one that hinders any discussion of events on Mars, is the lack of an absolute chronology. Although relative dating can be attempted from comparisons of crater counts and from stratigraphic relationships, we do not know the absolute age of any surface unit on the planet. Given the recent hiatus in planetary exploration (at least in the United States), it is unlikely that the situation will improve soon; absolute dating of events on Mars will probably require the return of samples for analysis on Earth.

In the absence of an absolute chronology, it is not surprising to find a lack of unanimity in discussions of when major events left their imprints on the surface. One extreme view is that Mars today is pretty much a dead planet, and that most of the geologically important events took place in the first billion years of its history. Carr's own view is less extreme. He suggests that although major geologic events, including the formation of most channels, were concentrated in the first half of the planet's history, important geologic activity has continued into the present. For example, volcanism appears to have extended throughout the life of the planet, and although Viking did not observe a single eruption it is highly unlikely that, volcanically, Mars is a dead planet.

Carr emphasizes that the study of surface features provides important clues to the evolution of the planet's interior and to the history of its atmosphere. There are geological indications that at some time the atmosphere of Mars was thicker than it is today, and there is ample evidence that large amounts of volatile material (presumably mostly H₂O) are frozen in the subsurface and in the polar regions. It is these reservoirs of volatiles that have been implicated in various models of climatic change. Although there remains a lively debate about the timing, extent and causes

of climate changes on Mars, the post-Viking consensus is that the planet never had an atmosphere as thick, or as moist, as that of Earth.

While the bulk of the 232 pages is devoted to surface geology — craters, volcanoes, canyons, chaotic terrain, channels, polar deposits and so on — also included are up-to-date summaries of our knowledge of the planet's interior and atmosphere. The book concludes with a contribution by Harold Klein (one of the biologists on the Viking team) reviewing the results of Viking's search for life, and a short chapter on Phobos and Deimos.

The casual browser will no doubt be seduced by the book's beautiful pictures, but the text is intended for serious readers. I found occasions where the inclusion of a few more key maps would have clarified the discussion, but in general the text is meticulously prepared. The few errors are clearly slips of the pen — for example,

Schiaparelli and not Antoniadi drew canals on Mars in 1877; and it was Mariner 4, and not Mariners 6 and 7, that made the first accurate measurements of atmospheric pressure.

While this is a Viking view of Mars, it would be unfair to call it Carr's view of Mars. The author has gone to great lengths to provide an unbiased description of what is in the pictures and a summary of major current interpretations. A reader looking for a simplistic approach which "explains" everything on Mars may be disappointed occasionally, but the serious student will appreciate Carr's painstaking effort to describe fairly the continuing debate about the many perplexing things that Viking saw and measured. *The Surface of Mars* is that rare beast, a book about Mars which does justice to its subject. □

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studies with little supportive enzymology, to the phenolic compounds where the majority of enzymes in some pathways have been purified and characterized, and the regulation of enzyme levels and of the flux through the pathway have been studied in detail. The highly successful studies of flavonoid biosynthesis from phenylalanine illustrate what is being achieved using modern methods, and show how investigation of what biochemists of other persuasions may regard as obscure compounds can yield information of wide significance.

The *Treatise* (the first three volumes of which have been reviewed in *Nature* already — 292, 783;1981) succeeds in a number of ways. It is a valuable work of reference; the individual volumes are well indexed and there are cross-references within and between volumes. It is relatively easy to track down specific topics in spite of the lack of a cumulative index for the work as a whole. In addition the deliberate overlaps — photorespiration, for example, is explicitly covered in Vols 2 (on metabolism and respiration) and 8 (photosynthesis) — means that each volume is self-contained and can be used as a text in its own right.

As an illustration, Vol.5 deals with amino acids (non-protein amino acids are covered in Vol.7). Besides coverage of individual families of compounds, there are chapters on physical and chemical properties, nitrogen fixation, ammonia and sulphate assimilation, transport of nitrogen compounds and the effects of environmental stress on amino acid accumulation.

For beginners, the authoritative accounts of well-investigated topics will facilitate entry into the appropriate fields. On the other side of the coin, the clear signposts to gaps in our knowledge should help identify attractive problems worthy of investigation. For these to be recognized, however, it is important to emphasize the biological context; plant biochemistry (and biophysics) must be seen in relation to the study of plants as well as of molecules. The mutual reinforcement between developmental, physiological and molecular approaches has already been fruitful in many areas. It is perhaps an inevitable weakness that the editors in concentrating on biochemistry *per se* and employing over one hundred authors, have not been uniformly successful in showing why the biochemistry is worth doing. The first chapters in Vol. 7 do, however, attempt this type of integration in a fairly systematic way.

Overall the *Treatise* is much to be recommended. It is an unfortunate — and unavoidable — paradox that many of the best chapters which deal with active and expanding topics will also go out of date most rapidly. □

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The patchwork of plant biochemistry

Philip Rubery

The Biochemistry of Plants: A Comprehensive Treatise. Eight-volume set. Editors-in-chief P.K. Stumpf and E.E. Conn. (Academic: 1980–1982.) Eight-volume set £303.60, \$480. Single volumes also available.

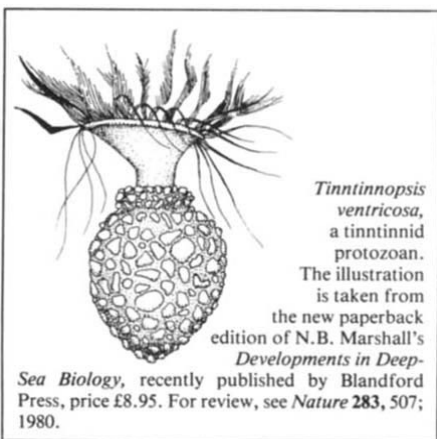
A COMPREHENSIVE treatise on plant biochemistry — some two million words and twenty-five thousand literature citations. What picture of the current state of plant biochemistry emerges from this mountain of paper, and how will future progress in the field be influenced?

Carbohydrate metabolism in its broadest sense — including photosynthesis, photorespiration, gluconeogenesis, energy storage and utilization, and the biosynthesis of structural polymers — is perhaps the most developed area of plant biochemistry and is well served by several volumes of the *Treatise*. Here, by and large, there are hard data on specifically botanical systems. However, research in

plant biochemistry is notoriously patchy and it is rather depressing to find so many chapters on aspects of primary metabolism which deal almost exclusively with data from animals and microorganisms. This is no fault of the authors (who commonly offer routine apologia), but rather is a consequence of absence of information from plants or, more often, of a hotch-potch of superficial and fragmented investigations from which a connected account cannot be constructed. It is to be hoped that such chapters, which mostly make the best of a bad job, will stimulate efforts to improve our understanding of metabolic processes in plants.

That this need not be an empty comparative exercise is shown by Vol. 4, on lipids, where the rapid progress in unravelling the distinctive features of plant systems is excellently covered. In contrast, much of the volume on proteins and nucleic acids (Vol. 6) is rather disappointing in that it is heavily orientated towards non-plant material in the general chapters on macromolecule biosynthesis. Inevitably parts of this volume will date rapidly and recent progress in plant molecular biology already gives it rather an old-fashioned look. It is noticeable that there is a greater sense of vitality when individual plant topics are dealt with (e.g. proteases and their inhibitors, plant tumours, plant viruses) than when the basic biochemistry is reviewed.

Volume 7 on secondary plant products covers an interesting cross-section of subjects. It reveals, for different classes of compounds, situations ranging from structural information accompanied by biogenetic hypotheses, through tracer



Tinntinnopsis ventricosa, a tinninnid protozoan.

The illustration is taken from the new paperback edition of N.B. Marshall's *Developments in Deep-*

Sea Biology, recently published by Blandford Press, price £8.95. For review, see *Nature* 283, 507; 1980.

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